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HEREDITAS,

a periodical devoted to the publication of original research in genetics and cytology, is published by the MENDELIAN SOCIETY of Lund, Sweden. All contributions are written in English, German or French, illustrated, when necessary, with adequate text figures and plates. The current volume (XXXVII) will contain about 500 pages, issued in four numbers.

Subscriptions should be sent to the Editor. Price *Twenty-five* (25) *Swedish Kronor per volume* (postage free). Most back volumes are still available. The price of Vols. I—X is 10 Swedish Kronor per volume, of Vols. XI—XII and XV—XXIII, 15 Sw. Kr., of Vols. XXIV—XXV, 20 Sw. Kr., and of Vols. XXVI—XXXVI, 25 Sw. Kr. Postage free. (Vols. XIII and XIV are out of print.)

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3 Adelgatan, LUND, SWEDEN.

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SPONTANEOUS AND X-RAY INDUCED GYNANDROMORPHS IN DROSOPHILA MELANOGASTER

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I. INTRODUCTION.

THE present paper contains results of several experiments on the production of gynandromorphs in *Drosophila melanogaster*. A description of different types of gynandromorphs produced in some of the series (series A, B, C, D) was made in an earlier paper (BONNIER, LÜNING and PERJE, 1949), but no frequencies were given on that occasion. In the present article several more experimental series will be described. In some of these, gynandromorphs are produced after x-ray treatment of males or of females, in other series (control series) a study is made of the spontaneous occurrence of gynandromorphs. Some considerations are also made on the production of hyperploid males.

The experiments were made in 1949 and 1950. During that time the technique used was changed in so far as in later series it was made possible to record the very days when those eggs were laid (= fertilized) from which gynandromorphs emerged.

All cultures, experimental and stocks, were kept in incubator at $25 \pm 1°$ C. Irradiations were made weekly at the Radiophysical Institute by using a Siemens Stabilivolt apparatus of 155 kilovolt with 1 mm Al.

Within series A, B, C, D we used a wild type and a *y w sn* stock; in all other series the two stocks *w sn* and *y f5 car*.

II. CLASSIFICATION OF GYNANDROMORPHS.

A gynandromorph is here defined as an animal, part of the cells of which have female and part male chromosomal equipment. From this definition it follows that not all gynandromorphs necessarily show their gynandromorph nature. By using suitable recessive marker genes and looking for gynandromorphs in a heterozygous F_1 progeny, the female parts are wild type whereas male parts show all or part of the recessive characters. An animal which is female and wild type, except on a very

small spot showing the recessive characters, may be a gynandromorph but may also be produced by somatic segregation, somatic crossing over or something similar, thus being a female. Furthermore, an animal which is female and wild type, except some part not including secondary sexual characters where it shows *one* of several possible characters, may also be a fractional mutant female. With these considerations in mind we classify the observed mosaics with regard to their possible gynandromorph nature in 3 types:

(1) Certain gynandromorphs. — Male on some or all of the secondary sexual characters (sex combs, wing size, coloration of 4th and 5th tergites, external genitalia) on which they show marker gene characters.

(2) Probable gynandromorphs. — Not male on secondary sexual characters but showing at least two marker gene characters on not too small an area (*viz.* including on thorax or head at least 4 bristles or 3 bristles and a surrounding area of hairs).

(3) Possible gynandromorphs. — Not male on secondary sexual characters but showing either two marker gene characters on an area smaller than that required in (2) or only one marker gene character on an area of any size.

To these 3 types we have had to add individuals which are sexually morphologically abnormal but which are not classifiable in any of the above 3 types. These individuals are infrequent and are not specified in the tables.

The possibility of detecting gynandromorphs depends much on the ability of the observer, and the possibility of overlooking gynandromorphs with minute male spots is always great. We know that differences in observational skill were to be found among our co-workers, but it would take too much space to specify the figures from each of them. All conclusions concerning frequency differences hold, however, good even when made on an intra-observer basis.

III. CONTROL SERIES.

The results of the control series are shown in Table 1. It is generally agreed that in spontaneous occurrence of gynandromorphs there is an equal chance for elimination of the maternal as for elimination of the paternal X-chromosome. MORGAN and BRIDGES (1919) found, for instance, 15 gynandromorphs of each kind, and PATTERSON and STONE (1938) report 35 paternal and 25 maternal eliminations. This is in good

TABLE 1. *Spontaneous gynandromorphs from crosses w sn ♀ × y f car ♂ and y f car ♀ × w sn ♂. Females and males were virgin at mating and remained together during the whole of the egg laying period. The males were, at mating, of a maximum age of 3 days.*

Series	Date of experiment	Cross	Age of females, days at mating	Total number of females and gynandr. in progeny	Number of gynandr. due to elimination of						Rate per 10^4			
					maternal X			paternal X			♂-type 1, 2		♂-type 1, 2, 3	
					1	2	3	1	2	3	mat.	pat.	mat.	pat.
N	July—Nov. 1949	*w sn* ♀ × *y f car* ♂	0,5 ± 0,5	116721	8	5	1	11	1	6	1,11	1,03	1,20	1,54
Q	Nov. 1949	» × »	7,5 ± 0,5	6542	1	—	—	2	1	—	1,53	4,59	1,53	4,59
Ax	July—Sept. 1950	» × »	7,5 ± 0,5	98142	13	6	—	5	3	2	1,94	0,82	1,94	1,02
Total		» × »		221405	22	11	1	18	5	8	1,49	1,04	1,54	1,40
Az	July—Sept. 1950	*y f car* ♀ × *w sn* ♂	0,5 ± 0,5	88407	10	—	—	13	2	—	1,13	1,70	1,13	1,70
At	May—Aug. 1950	» × »	7,5 ± 0,5	93004	7	2	1	28	4	—	0,97	3,44	1,08	3,44
Total		» × »		181411	17	2	1	41	6	—	1,05	2,59	1,10	2,59
Grand total				402816	39	13	2	59	11	8	1,29	1,74	1,34	1,94

Deviations from 1 : 1 proportion between maternal and paternal eliminations

	♂ types 1, 2		types 1, 2, 3	
	χ^2	P	χ^2	P
Cross *w sn* ♀ × *y f car* ♂	1,79	0,10—0,20	0,14	0,70—0,80
» *y f car* ♀ × *w sn* ♂	11,88	< 0,001	10,88	0,001

Significance, P, of differences between the two crosses

	♂ types 1, 2	types 1, 2, 3
maternal eliminations	0,21	0,22
paternal »	< 0,001	< 0,01

Significance of difference between paternal eliminations, series Az *vs.* At, P = 0,02

Significance of difference between maternal eliminations, series N *vs.* Ax + Q, P = 0,18

agreement with our series in which *w sn* females were crossed to *y f car* males (Table 1). In the reciprocal cross, however, there is a very great preponderance of paternal eliminations. Now, as the females in the progeny of the two crosses are identical with respect to their genotype (chromosomal equipment), the difference may be supposed to be due, at least in part, to cytoplasmic differences. It is interesting to note (Table 1, bottom), that the difference between the proportions of maternal eliminations from the two types of crosses is without statistical significance whereas, in the case of paternal eliminations, the difference is of a good significance. It seems thus plausible that in different strains differences in the egg cytoplasm act differentially on the chance that chromosomes from entering spermatozoa will be eliminated during ontogeny.

A comparison is made between the ages of the females from series Ax and At (in both of which the females were mated at the same age, *viz.* 7,5 $\pm$ 0,5 days) when laying the eggs from which gynandromorphs emerged. Table 2 shows the averages and the significance. As seen, the

TABLE 2. *Comparison between series Ax and At (Table 1) with regard to average time, days, from mating to fertilization of eggs from which gynandromorphs emerged. (Included are gynandromorphs types 1 and 2 only.)*

Series	Cross	Average age of females when laying eggs from which gynandromorphs emerged	
		maternal	paternal
Ax	*w sn* ♀ × *y f car* ♂	6,3	7,6
At	*y f car* ♀ × *w sn* ♂	6,6	8,1

Significance, P, of differences
maternal *vs.* paternal eliminations 0,05—0,20 (about 0,08)
series Ax *vs.* At $>$ 0,20

eggs from which the paternal eliminations emerged were laid at an averagedly higher age than those from which the maternal eliminations emerged. The difference is not significant. However, in x-raying the females a longer time elapsed from irradiation to laying the eggs from which paternal eliminations emerged than to laying eggs giving rise to maternal eliminations (Table 2). This difference is of a better significance and, when the two facts are taken together, it might indicate some changes with ageing the females, presumably in the cytoplasm, which

to a higher degree act on the possibility of eliminations of the paternal than of the maternal X-chromosome.

By comparing the rates of paternal eliminations in series Az and At it is found that the probability that the difference is due to chance is only 0,02 (Table 1, bottom). It does not seem likely that environment is the cause of this difference which therefore would indicate that the age of the mother influences the rate of eliminations of *w sn* chromosomes in the cytoplasm of our *y f car* strain. The corresponding difference between the rates of maternal eliminations in series N *vs.* series Q + Ax (where there likewise is a higher rate in older females, series Q + Ax, than in younger females, series N) is not significant (Table 1, bottom). It should, however, be remembered that series N and Q were made one year earlier than Ax.

IV. MALE IRRADIATION SERIES.

Two different types of male irradiation series were made, one in 1949 and one in 1950. The later of these two series will be described first.

1. DOSE-RATE DEPENDENCE.

A. *Gynandromorphs due to elimination of the irradiated chromosome.* — Starting from ideas of MULLER (1940) and PONTECORVO (1942), a mode of production of gynandromorphs in the progeny of irradiated males was previously outlined (BONNIER, LÜNING and PERJE, 1949). It was supposed that after a break in the unsplit spermatozoon X-chromosome the break remains unhealed and unrestituted until the beginning of active chromosomal movements in conjunction with splitting and fertilization. If one of the chromatids then restitutes and one is lost and the zygote is viable a gynandromorph ensues. It is of course impossible to prove this assumption but, as it involves the hypothesis of single breaks, the rate should be linearily dependent upon dose. In order to study this, three doses proportioned as 1 : 3 : 5 were used. The lowest was somewhat below 800 r and the two others consequently somewhat below 2400 r and 4000 r, respectively. It should be noted that the control series Ax, Az, At (Table 1) were made simultaneously with these irradiation series. As all females used in the x-ray series were $7{,}5 \pm 0{,}5$ days old at mating the controls most appropriate for a study of linearity are: series Ax in case of the cross *w sn* female × *y f car* male and series At in the case of the cross *y f car* ♀ × *w sn* ♂. The corresponding linear regression lines are in Figs. 1 and 2 denoted by *a*. We have also studied

the linear regression lines in which for the zero dose the averages of series N, Q and Ax, respectively, the averages of series Az and At are used. These lines are in the figures denoted by *b*. In all these computations we have made use of the frequencies of paternal eliminations of all three ♀ types 1, 2, and 3. The significance of the deviation from

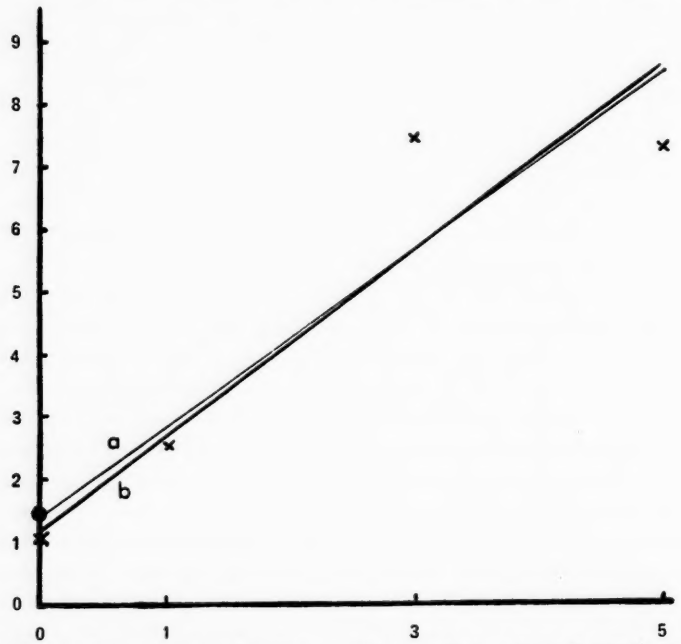

Fig. 1. Rates of gynandromorphs after crossing *w sn* ♀ to *y f car* ♂ (Table 3). For dose 0 the cross corresponds to series Ax and the dot to the total of series N, Q, and Ax (Table 1). *a* and *b* are the two corresponding linear regression lines. Horizontal scale, dose (1 = somewhat below 800 r); vertical scale, rate per 10^4.

the regression lines is tested by comparing the observed frequencies with those expected from the regression. It it seen (Table 3, bottom) that the fits are satisfactory. This means that there is nothing which, so far, is contradictory to the hypothesis that x-ray induced gynandromorphs are due to single breaks.

B. *Hyperploid males.* — From the cross *w sn* ♀ × *y f car* ♂ a few sterile *sn* males emerged and from the cross *y f car* ♀ × *w sn* ♂ sterile *f car* males occurred. The frequencies are found in Table 4. Such males are supposed to be due to a piece of the irradiated X-chromosome having

been translocated, the resulting males then having this piece in addition to their complete maternal X. When, in the cross *w sn* ♀ × *y f car* ♂, *sn* males occur, the piece comes presumably from the distal end of the *y f car* chromosome and includes the w^+ gene. This is a rather long piece and the majority of such males probably die before hatching. The low

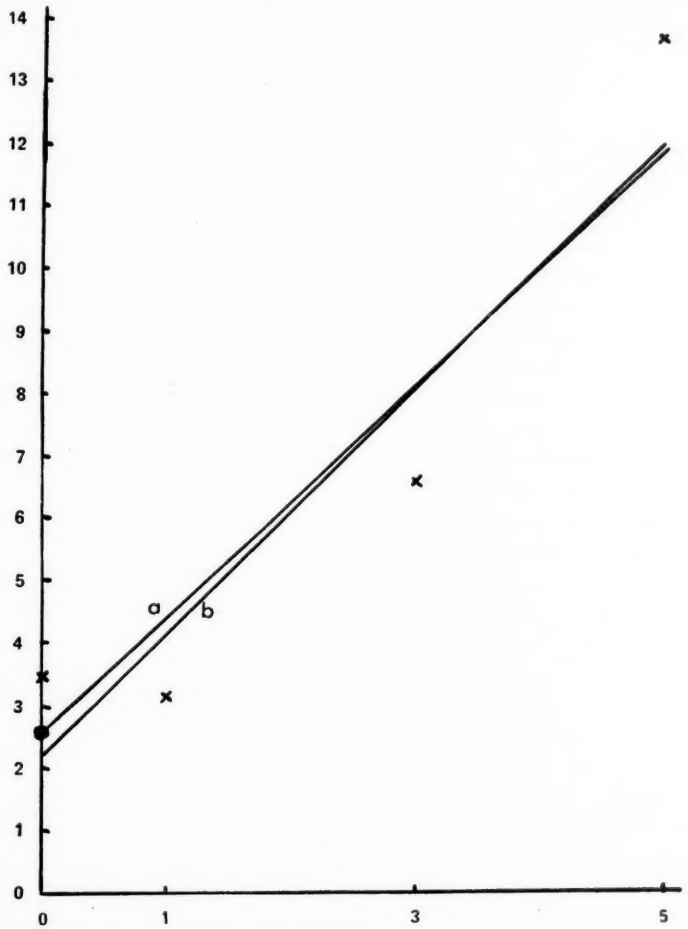

Fig. 2. Rates of gynandromorphs after crossing *y f car* ♀ to *w sn* ♂ (Table 3). For dose 0 the cross corresponds to series At and the dot to the total of series At and Az (Table 1). *a* and *b* are the two corresponding linear regression lines. Horizontal scale, dose (1= somewhat below 800 r); vertical scale, rate per 10^4.

TABLE 3. *Gynandromorphs produced in the progeny of irradiated males and untreated females. Females and males were virgin at mating and remained together for the whole of the egg laying period. The males were at mating of a maximum age of 3 days. They were mated immediately after irradiation. The females were at mating 7,5 ± 0,5 days of age. Three doses, proportioned as 1 : 3 : 5 (equal intensities) were given. Dose »1» was somewhat below 800 r. All experiments were conducted during the time from March to October, 1950.*

Series	Cross	Dose	Total number of females and gynandr. in the progeny	Number of gynandr. due to elimination of						Rate per 10^4			
				maternal X			paternal X			♂ type 1, 2		♂ type 1, 2, 3	
				♂ type			♂ type						
				1	2	3	1	2	3	mat.	pat.	mat.	pat.
Ak	*w sn* ♀ × *y f car* ♂	»1»	50518	4	—	—	11	1	1	0,79	2,38	0,79	2,57
Am	» × »	»3»	32322	3	1	—	19	3	2	1,24	6,81	1,24	7,43
Ao	» × »	»5»	26318	2	—	—	15	2	2	0,76	6,46	0,76	7,22
Al	*y f car* ♀ × *w sn* ♂	»1»	57863	8	—	3	17	1	—	1,38	3,11	1,90	3,11
An	» × »	»3»	34967	1	—	1	23	—	—	0,29	6,58	0,57	6,58
Ap	» × »	»5»	24843	4	—	—	31	2	1	1,61	13,28	1,61	13,68

Linear regression lines and significance of deviations of observed rates of paternal eliminations (types 1, 2, 3) from expected rates according to regression.

Cross *w sn* ♀ × *y f car* ♂

for dose 0: series Ax (Table 1) $Y = 1{,}22 + 1{,}46\,x$ (*a* Fig. 1) $\chi^2 = 2{,}82$ P 0,5—0,7
» » »: » N + Q + Ax (Table 1) $Y = 1{,}45 + 1{,}40\,x$ (*b* Fig. 1) $\chi^2 = 2{,}51$ P 0,5—0,7

cross *y f car* ♀ × *w sn* ♂

for dose 0: series At (Table 1) $Y = 2{,}56 + 1{,}85\,x$ (*a* Fig. 2) $\chi^2 = 5{,}43$ P 0,2—0,3
» » »: » At + Az (Table 1) $Y = 2{,}21 + 1{,}95\,x$ (*b* Fig. 2) $\chi^2 = 4{,}15$ P 0,3—0,5

frequencies (Table 4) are thus explained. In the cross *y f car* ♀ × *w sn* ♂ the translocated piece may only involve the short distance from the terminal end to the y^- locus inclusive, and the vitality of such males is fairly good. We have, accordingly, observed larger frequencies of hyperploid males in this cross than in the reciprocal one. The rates per 10^4 given in Table 4 are computed on the basis of the same totals as those shown in Tables 1 and 3, *i. e.* on the total number of XX individuals and not on the totals of XY individuals. It is probably without importance on which of these two totals the rates are based, and so we used the more convenient way.

TABLE 4. *Hyperploid males from same experiments as those shown in Tables 1 and 3.*

Dose	Cross	*sn* males number	*sn* males per 10^4 of totals in Tables 1 and 3	Cross	*f car* males number	*f car* males per 10^4 of totals in Tables 1 and 3
0	*w sn* ♀ × *y f car* ♂	0	0,00	*y f car* ♀ × *w sn* ♂	0	0,00
»1»	» × »	2	0,40	» × »	11	1,90
»3»	» × »	1	0,31	» × »	12	3,43
»5»	» × »	5	1,90	» × »	27	10,86

Regression line and significance of deviation of observed frequencies from those expected according to regression, for *f car* males. (For dose 0 the total of series Az + At, Table 1, is used.)

$Y = 0{,}12 + 0{,}90\, x^{3/2}$ $\chi^2 = 7{,}8$ $P = 0{,}10$

As translocations involve at least 2 breaks the dose-rate dependence ought to be a quadratic one. But since several of the resulting chromosomal combinations lead to lethality it is generally supposed that the rate is proportional to $(\text{dose})^{3/2}$. (*Cf.* MULLER, 1940.) The hyperploid males from the cross *w sn* ♀ × *y f car* ♂ (Table 4) are too few to permit an analysis of dose-rate dependence but for the reciprocal cross such an analysis is possible. It is found (Table 4, Fig. 3) that the regression line $Y = 0{,}18 + 0{,}90\, x^{3/2}$ gives a fairly good fit. When making a χ^2-test in the same way as in the preceding cases one gets a probability, P, of 0,10. This value is, however, certainly too low since the expected frequency for dose 0 is 2 and that for dose 1 is 6. For such low expected frequencies χ^2 always exaggerates the discrepancy between observed and expected, and, in the present case, the sum of χ^2 corresponding to these two doses is 6,17 out of a total χ^2 of 7,80.

That a straight line does not fit the observed values is shown from a glance at Fig. 3. We may thus conclude that the hyperploid males occur after 2 or more breaks.

2. INFLUENCE OF DURATION FROM IRRADIATION TO FERTILIZATION AND OF AGE OF MALES.

In a preceding article (BONNIER, LÜNING and PERJE, 1949) descriptions were made of gynandromorphs which originated from mating untreated *y w sn* females to irradiated wild type males. In that paper no figures were given of the frequencies with which these gynandromorphs occurred. They are here summarized in Table 5. The males were given a dose somewhat less than 3200 r, or equal to dose »4» when

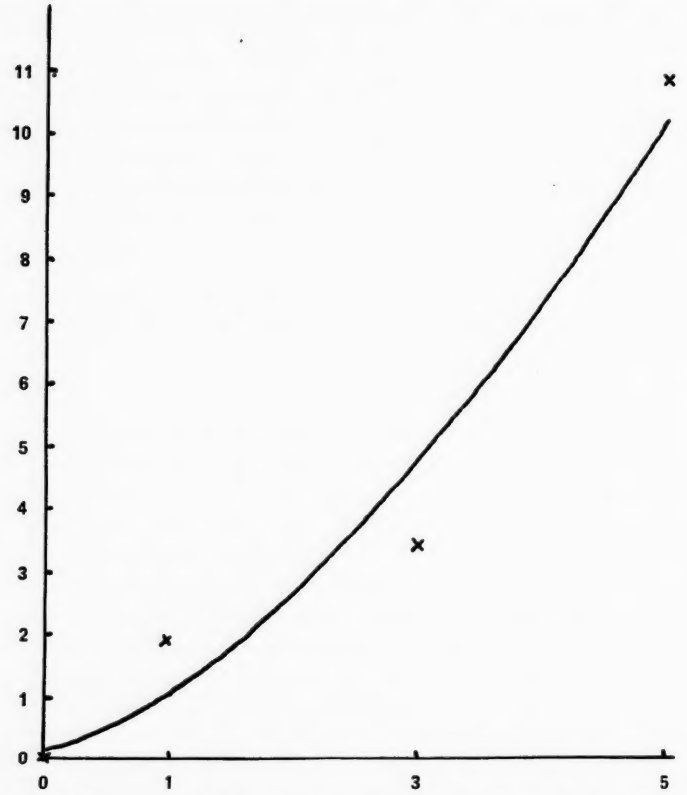

Fig. 3. Rates of hyperploid males after crossing *y f car* ♀ to *w sn* ♂ and curved regression line proportional to (dose)$^{3/2}$. Horizontal scale, dose (1 = somewhat below 800 r); vertical scale, rate per 10^4.

the same scale as in Tables 3 and 4 is used. All females were of a maximum age at mating of 3 days. Four parallel series A, B, C, D were made, the difference being the age at which the males were treated and/or the duration from irradiation to mating. After being kept unmated from birth, the males of series A were irradiated at an age of $7{,}5 \pm 0{,}5$ days and in series B at an age of $0{,}5 \pm 0{,}5$ days. After irradiation, these males were kept unmated for 4 to 5 days when they were mated in mass cultures to virgin females. These females were on the 7th day from irradiation transferred to fresh bottles, 4 to 5 per bottle and without males. In series C irradiations were made as in series A, and in

TABLE 5. *Progeny from crosses of untreated ywsn females to irradiated wild type males (dose »4» according to scale in Table 3). Rates of gynandromorphs due to elimination of paternal X-chromosomes and of hyperploid males. Experiments conducted Jan.—April, 1949.*

Series	Age of males, days, at irradiation	Approximate duration, days, from irradiation to fertilization	Gynandromorphs types 1	2	3	Total females and gynandromorphs	Rate per 10^4 of gynandromorphs types 1 and 2	Hyperploid males *w sn*	*sn*	rate per 10^4
A	$7{,}5 \pm 0{,}5$	7—14	24	7	—	37786	8,20	29	2	8,20
B	$0{,}5 \pm 0{,}5$	7—14	27	5	—	46692	6,85	36	2	8,14
C	$7{,}5 \pm 0{,}5$	0— 7	26	4	—	46217	6,49	35	5	8,65
D	$0{,}5 \pm 0{,}5$	0— 7	37	12	1	43264	11,32	22	1	5,32

Significance, P, of differences	gynandromorphs	hyperploids
Series A *vs.* D	0,16	0,12
» B *vs.* D	0,03	0,10
» C *vs.* D	0,02	0,06
» A + B + C *vs.* D	0,02	0,03

series D as in series B. Immediately after irradiation the males of series C and D were mated to virgin females, 4 to 5 of each sex per bottle, and remained with the females until the beginning of counts of the F_1 progeny. In all series progeny counts were made only once per bottle, usually on the 16th day from the start of the cultures. Taking the time from egg-laying to hatching to be roughly 10 days, it follows from the design of the experiments that the duration from irradiation to fertilization of eggs from which progeny was counted varied in series A and B roughly from 7 to 14 days and in series C and D from 0 to 7 days.

As gynandromorphs in the present series due to elimination of the untreated maternal X-chromosome are necessarily much more difficult to detect, Table 5 includes only such gynandromorphs which are due to elimination of the treated paternal X-chromosome.

There occurred in the progeny also a limited number of sterile hyperploid *w sn* and *sn* males (Table 5). As in the preceding case, these males contain, besides the maternal X, an extra piece of the distal end of the treated X. Table 5 shows now that the rates per 10^4 are more or less similar for the three series A, B and C. Series D, on the other hand, has a considerably higher rate of gynandromorphs and a considerably lower rate of hyperploid males. A statistical analysis shows that the differences in rates of gynandromorphs, B *vs.* D, C *vs.* D, and of the

pooled A + B + C *vs.* D is of a rather strong significance. And also that the difference in rates of hyperploid males is of a rather good significance in the case of comparing the pooled A + B + C *vs.* D. From Table 5 it thus follows that increase of time from irradiation to fertilization (comparison of series D and B) as well as increase of males' age at irradiation (comparison of series C and D) decreases the rate of gynandromorphs and increases the rate of hyperploid males. Thus, both of these two causes, length of time from irrradiation to fertilization and age of male at irradiation, act as varying causes of the rates in question. If the explanation of the production of gynandromorphs from irradiated males is valid it is possible to imagine that the two parts of the chromosome are not absolutely immobile relative to each other after break. It seems, then, possible to assume that the distance between the broken ends should increase with lapse of time from breakage. And, if it were possible to register the distance between the ends at the moment when the active movements of the chromosome begin and the restitutable ends are restituted, *i. e.* at a moment correlated with the moment of fertilization, it would, according to this assumption, presumably be found that restitution occurs more frequently when this distance is short and loss of distal ends more frequently when the distance is long. Or, in other words, when fertilization takes place shortly after breakage there would be found a higher rate of restitutions than later. In a transitory stage, then, the cases of restitution of one and loss of one of the two chromatids would have their maximal frequency. If this transitory stage falls within the first week after irradiation the decrease in rate of gynandromorphs in series B as compared with series D would be explained.

With regard to the hyperploid males it could be imagined that, just as the distance between broken ends in the X-chromosome increases, the same would be the case with ends of broken autosomes permitting a larger number of translocations. This would explain the increase in the rate of hyperploid males in series B as compared with series D.

When making the present experiments it was observed that in the culture bottles of series D there were usually larger progenies than in those of the other three series. This was the reason why we undertook egg counts from crosses more or less corresponding to the present four series, the results of which were recently published (BONNIER and LÜNING, 1950). There we found that the rate of dominant lethals increases with ageing the males. DEMPSTER (1941) and STRÖMNÆS (1949) arrived at the same conclusion. The latter author assumed that this was wholly due to ageing the spermatozoa and not to the males' age as such.

The data of BONNIER and LÜNING (1950), however, indicate that the males' own age is of importance. It was, therefore, supposed that the chromosomes change physiologically so as to make the moving apart of the broken ends more speedy in older than in younger males. In the present experiments a comparison of series C and D shows an effect of the males' age at irradiation. This may also be assumed to be due to a physiological change in the chromosomes so that the increase of the distance between the broken ends takes place at a higher speed in older than in younger males. If this is so, the fact that series A does not show a lower rate of gynandromorphs or a higher rate of hyperploids than series B and C indicates that the movability relative to each other of the broken ends is limited in time and/or space.

V. FEMALE IRRADIATION SERIES.

In their basic work on gynandromorphs induced by x-ray irradiation PATTERSON (1931) and PATTERSON and STONE (1938) found that when irradiated females are mated to untreated males there occur gynandromorphs due to elimination of the paternal (untreated) as well as of the maternal (treated) X-chromosome. PATTERSON concludes that when female germ cells are treated there is probably an indirect effect of x-rays on the paternal X operating through the cytoplasm. PATTERSON and STONE found 77 gynandromorphs of which 36 were due to elimination of the maternal and 41 to elimination of the paternal X, indicating that eliminations of maternal and paternal X-chromosomes are produced with about equal frequencies. As it seemed possible that this very interesting phenomenon of indirect action could be influenced by differences in age of females and of differences in length of time from irradiation to fertilization the following experiments were started.

All males used in the present experiments were of a maximum age at mating of 24 hours. After mating, the treated females remained with their mates. The technique used made it possible to check (except in series J) the ages of the females at laying the eggs from which gynandromorphs emerged and also of the duration from irradiation to laying of these eggs. Four series of crossing *w sn* females to *y f car* males (series J, P, L, M) and four series of the reciprocal cross (series Ac, Ad, Ae, Af) were made (Table 6). The different arrangements in these series are indicated in the table, and some statistical comparisons are made at the bottom of the table. As seen, only two of the comparisons show statistically clear differences. These are: the differences in the rates of paternal

TABLE 6. *Gynandromorphs produced in the progeny of irradiated females and untreated males. Females and males were virgin at mating and remained together during the whole of the egg-laying period. The males were at mating of a maximum age of 1 day. The doses refer to scale of Table 3. Cross in J, P, L, M w sn ♀ × y f car ♂, in Ac, Ad, Ae, Af y f car ♀ × w sn ♂.*

Series	Date of experiment	Age of females days at		Dose	Total number of females and gynandromorphs in progeny	Gynandromorphs due to elimination of						Rate per 10^4			
						maternal X			paternal X			♂ type 1, 2		♂ type 1, 2, 3	
						♂ type			♂ type						
		irrad.	mating			1	2	3	1	2	3	mat.	pat.	mat.	pat.
J	May—July 1949	0,5 ± 0,5	0,5 ± 0,5	»3»	62704	6	1	—	6	1	2	1,12	1,12	1,12	1,28
P	July—Oct. 1949	0,5 ± 0,5	7,5 ± 0,5	»3»	78703	10	—	1	18	1	—	1,27	2,41	1,40	2,41
M	July—Oct. 1949	7,5 ± 0,5	7,5 ± 0,5	»3»	65754	3	2	—	14	—	3	0,76	2,13	0,76	2,59
L	Nov.—Dec. 1949	7,5 ± 0,5	7,5 ± 0,5	»1,5»	59954	2	—	1	15	—	5	0,33	2,50	0,50	3,34
Ac	Dec. 1949-Febr. 1950	7,5 ± 0,5	7,5 ± 0,5	»1,5»	31311	5	—	4	5	1	3	1,60	1,92	2,87	2,87
Ad	»	10,5 ± 0,5	10,5 ± 0,5	»1,5»	30044	6	—	2	5	1	—	2,00	2,00	2,66	2,00
Ae	»	4,5 ± 0,5	4,5 ± 0,5	»1,5»	25006	2	1	—	5	2	—	1,20	2,80	1,20	2,80
Af	»	0,5 ± 0,5	0,5 ± 0,5	»1,5»	28929	4	1	2	3	—	—	1,73	1,04	2,42	1,04

Significance, P, of difference between rates of gynandromorphs in controls (Table 1) *vs.* irradiation series

Cross		♂ types 1, 2	♂ types 1, 2, 3
w sn ♀ × *y f car* ♂	maternal	0,07	0,08
	paternal	0,008	0,004
y f car ♀ × *w sn* ♂	maternal	0,18	0,02
	paternal	0,03	0,20

Significance, P, of difference between rates of maternal *vs.* paternal eliminations in irradiation series

Cross *w sn* ♀ × *y f car* ♂	0,0004	0,00004
y f car ♀ × *w sn* ♂	0,64	0,79

eliminations from treated and untreated series in the cross *w sn* ♀ × *y f car* ♂, and the difference in the irradiation series of the same cross between rates of maternal and paternal eliminations. In the two comparisons of control *vs.* irradiation in the cross *y f car* ♀ × *ws n* ♂ there are found widely different levels of significance when, on the one hand, using the gynandromorph types 1 and 2 only and, on the other, using the three types 1, 2 and 3. As explained in the description of the classification of gynandromorphs, there is certainly, among type 3, a number which should not be entered among the gynandromorphs. Consequently, one must be very cautious in drawing, from our figures, any conclusion of an increasing effect of x-rays on the frequency of maternal eliminations. In this respect we have thus not been able to confirm with certainty the findings of PATTERSON (1931). An increasing effect in the cross *y f car*♀ × *w sn* ♂ on the paternal eliminations is also very doubtful. It should be added that an analysis of the figures of Table 6 does not show any significant difference from changes of dosage (comparison of series M and L) or between series with different ages of the females at mating or irradiation.

However, when comparing the average duration from irradiation to laying of the eggs from which gynandromorphs emerged we find that this is longer for paternal than for maternal eliminations. The comparisons made in Table 7 include all series in which the females were mated immediately after irradiation (excl. series J where the records did not permit such a comparison). The longer duration for paternal than for maternal eliminations may be a consequence of a higher average age of the females when laying eggs from which paternal eliminations occur than when laying eggs from which maternal eliminations occur, as was shown to be probable for controls (Table 2). But it may possibly also be a direct effect of irradiation. Though we have not been able to confirm PATTERSON's findings that irradiation of females increases the pro-

TABLE 7. *Comparison between the two types of crosses in the female irradiation series with regard to average duration, days, from irradiation to laying of the eggs from which gynandromorphs emerged. (Included are gynandromorphs types 1 and 2 only.)*

Series (Table 6)	Cross	Duration	
		maternal	paternal
M, L	*w sn* ♀ × *y f car* ♂	4,9	8,6
Ac, Ad, Ae, Af	*y f car* ♀ × *w sn* ♂	6,7	8,3

Significance, P, of difference between maternal *vs.* paternal eliminations 0,05—0,01

portion of maternal eliminations we may now discuss how, if real, such eliminations may be produced.

Paternal eliminations after male irradiation were supposed to be due to single breaks and this was substantiated by the linear dose-rate relationship (Figs. 1 and 2). Now, breaks occur in female chromosomes after female irradiation just as in male chromosomes after male irradiation. But a directly corresponding mode of production of maternal eliminations after female irradiation is probably not possible. It is known (HUETTNER, 1924) that, before entry of the sperm, the maturation of the female germ cells proceeds only up to first metaphase. Thus, a break in a female must take place in a chromosome prior to gamete formation. The resulting gamete would, therefore, either contain a wholly restituted chromosome leading to a normal female, or, which is much less probable, a chromosome which is broken and lacks the broken-off end, thus without possibility for later restitution leading to an XO male or, finally, a hyperploid male could be formed. In none of the cases would a gynandromorph be produced. It seems thus most probable that, if and when, gynandromorphs are produced by irradiating females they must be the result of indirect forces via the egg cytoplasm — and that this may be expected to be the case with regard to maternal as well as to paternal eliminations. Such an influence from the cytoplasm may be exerted on the movements of the chromosomes by disturbances in the karyokinetic forces presumably at the first cleavage. According to HUETTNER (1924), the two pronuclei form separate mitotic figures at the first cleavage, common mitoses not beginning until the second cleavage. If one of the X-chromatids in the female or male pronucleus is lost during the first cleavage one of the resulting nuclei will be XX and the other XO, thus giving rise to a gynandromorph. Now, it was found (Table 2) that eggs which produce maternal eliminations are laid at an earlier age of the females than eggs which produce paternal eliminations (non-significant difference) and that (Table 7) the corresponding is true for the duration from irradiation to egg laying (significant difference). If this is an effect of age itself one could possibly imagine that the disturbing changes in the spindle substances are more severe for chromosomes of spermatozoa entering eggs from older females than from younger ones or/and that the converse is true for maternal chromosomes, *i. e.* that the disturbances exerted on spindle-forming substances and/or on centromeres are more severe on chromosomes residing in younger females' eggs than in those of older ones. It should also be emphasized that the difference in response between maternal and paternal chromosomes may be cor-

related with the fact that the paternal chromosomes of the first cleavage mitosis have to move through the irradiated cytoplasm whereas the maternal chromosomes remain relatively more stationary.

We may summarize these considerations by making the following tentative assumptions which are in accordance with the figures in Tables 1 and 6. Irradiation of females produces disturbances in the karyokinetic mechanism of the cytoplasm. As a consequence the production of gynandromorphs is increased and this is most marked for paternal eliminations. In certain strains (*viz. w sn*, Table 1), however, the cytoplasm is of such a nature that in the first cleavage mitosis, it acts, already without irradiation, more severely on the movements of the male chromosomes than on the female chromosomes, at least when these chromosomes are »foreign» to the cytoplasm (*y f car*, Table 1). In such cases (Table 6) irradiation cannot exaggerate the disturbances and there will be no, or only an insignificant, increase in paternal eliminations.

Acknowledgements. — The authors are very much indebted to Professor R. SIEVERT and Dr. A. FORSSBERG of the Radiophysical Institute for their courtesy in facilitating the irradiation work; to Dr. A.-M. PERJE, Mr. J. HAMMARÉN, Mrs. S. HENNINGS, Mrs. G. DE LAVAL, Miss T. RIBBING, Mrs. B. ARNBERG, and Miss M.-L. BENDEL for their painstaking and most valuable help in the laboratory work; and to Professor T. CASPERSSON for his great kindness in still harbouring within his laboratories the Institute of Genetics during a time before the Institute's own localities were ready.

Our investigation was supported by the Swedish Natural Science Research Council.

SUMMARY.

(1) The present article describes experiments on the production of gynandromorphs after x-ray irradiation of male and female *Drosophila melanogaster*. It includes a study of spontaneous production of gynandromorphs and of the production of hyperploid males from x-rayed males. A total of 1.186.011 females and gynandromorphs was recorded in these experiments of which 575 were classified as gynandromorphs.

(2) The spontaneous rate of maternal and paternal eliminations were about equal in the cross *w sn* ♀ × *y f car* ♂, but in the reciprocal cross a great preponderance of paternal eliminations was found. It is

supposed that there are differences in the egg cytoplasm of the two strains which act differentially on the chance that chromosomes from entering spermatozoa will be eliminated during ontogeny. Eggs from which paternal eliminations emerge are laid at an averagedly higher age of the females than eggs from which maternal eliminations emerge. Though the difference is not in itself statistically significant, age differences in x-ray experiments indicate that age is really effective as a varying cause on chromosome elimination.

(3) The dose-rate relationship of paternal eliminations after x-ray irradiation of males is linear, supporting the hypothesis that gynandromorphs are due to single breaks in the male chromosomes.

(4) Increasing the duration from irradiating the males to fertilization of the eggs from which paternal eliminations emerge increases the rate of these gynandromorphs. It is supposed that this is a consequence of a moving apart of the broken chromosome ends. The males' age is also influential and this may be explained by supposing a physiological change with age in the speediness with which the ends move apart.

(5) The rate of hyperploid males after irradiating males is proportional to $(\text{dose})^{3/2}$. It is concluded that hyperploid males are due to 2 or more breaks.

(6) It has not been possible to confirm with certainty the view that irradiation of females increases the rate of maternal eliminations. It does, however, increase the rate of paternal eliminations in those crosses where the spontaneous rates of maternal and paternal eliminations are about equal, but not in those crosses where there is an excess of spontaneous paternal eliminations. It is supposed that the effect of irradiation is a disturbance of the karyokinetic mechanism of the egg cytoplasm which presumably acts on the movements of the chromosomes at the first cleavage mitosis.

LITERATURE CITED.

1. BONNIER, G. and LÜNING, K. G. 1950. X-ray induced dominant lethals in *Drosophila melanogaster*. — Hereditas XXXVI, 445—456.
2. BONNIER, G., LÜNING, K. G. and PERJE, A. M. 1949. Studies on x-ray mutations in the white and forked loci of *Drosophila melanogaster*. II. A study of the formation of gynandromorphs and other kinds of mosaics. — Hereditas XXXV, 301—336.
3. DEMPSTER, E. R. 1941. Dominant *vs.* recessive lethal mutations. — Proc. Nat. Acad. Sc. 27, 249—250.
4. HUETTNER, A. F. 1924. Maturation and fertilization in *Drosophila melanogaster*. — Jour. Morph. 39, 249—265.

5. MORGAN, TH. and BRIDGES, C. B. 1919. The origin of gynandromorphs. — Carnegie Inst. Publ. 278, 1—122.
6. MULLER, H. J. 1940. An analysis of the process of structural change in chromosomes of *Drosophila*. — Jour. Genet. 40, 1—66.
7. PATTERSON, J. T. 1931. The production of gynandromorphs in *Drosophila melanogaster* by x-rays. — Jour. Exp. Zool. 60, 173—211.
8. PATTERSON, J. T. and STONE, W. 1938. Gynandromorphs in *Drosophila melanogaster*. — The Univ. of Texas Publ. 3825, 1—67.
9. PONTECORVO, G. 1942. The problem of dominant lethals. — Jour. Genet. 43, 295—300.
10. STRÖMNÆS, Ö. 1949. The production of dominant lethals with x-rays in aged *Drosophila* sperm. — Genetics 34, 462—474.

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MUSTARD GAS AND GYNANDROMORPH PRODUCTION IN DROSOPHILA MELANOGASTER

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INTRODUCTION.

AFTER MULLER's discovery of the mutagenic effects of X-rays it was not long before it was also found that the frequency of gynandromorphs increased after irradiation of males. This was studied in *Drosophila melanogaster* by PATTERSON (1931) and PATTERSON and STONE (1938). They also showed that after irradiation of females there was an increase not only in the rate of gynandromorphs due to elimination of the maternal X-chromosomes but of the paternal as well. They found equal rates for the two kinds of eliminations.

Through AUERBACH's (1943) discovery of the mutagenic effect of mustard gas and its potency in producing chromosome eliminations (AUERBACH, 1947) a new field was opened for investigations on chromosome eliminations.

As it was of great interest to compare the production of gynandromorphs after treating *Drosophila melanogaster* females with X-rays (BONNIER and LÜNING, 1951) a series of experiments with mustard gas have been made. The present paper deals with the results.

METHODS.

In the treatments, sulphur mustard gas, placed at our disposal by Försvarets Forskningsanstalt, Stockholm, was used. At an early stage of the experiments the difficulties of checking the dosage were obvious. A method was then chosen, which was technically simple and easy to repeat. The apparatus was an ordinary culture bottle, a milk bottle of $^1/_3$ litre, a rubberstopper and a capillary with the diameter of about 0,1 mm. With aid of the capillary a small quantity of mustard gas was placed inside the bottle. The flies were transferred and the rubberstopper was put in. Afterwards the area of the bottle, where the mustard gas was placed, was heated until it had evaporated. A lighted match gave

the necessary temperature. The bottle was shaken now and then during 4 minutes whereupon the flies were transferred to a fresh bottle and treated with a weak current of compressed air for about one minute. Thereafter they were transferred to another fresh bottle. Through these changes of bottles the flies are brought into an atmosphere which can be supposed to be practically free from mustard gas. So the time of treatment is fairly well-defined. After the treatment the flies stayed in the last bottle about 2 hours before they were transferred to the egg collector together with untreated flies of the opposite sex.

The egg collector consists of a preserving jar with a wooden cover containing a removable Petri dish with agar corn meal food. The Petri dish was changed daily except Sundays during 14 days and the agar plate was divided into smaller pieces according to the supply of eggs. Each piece was put in a culture bottle placed in an incubator at 25° C during 14 days. F_1 daughters were inspected with great care and aberrations were collected and registered. Most mutations were mated for closer examination. Those aberrations, which contained male secondary sex characters in the aberrant area, were classified as gynandromorphs, type 1, or areas with at least two mutant characters, type 2. For further details, see BONNIER and LÜNING (1951).

EXPERIMENTS.

In these experiments the following stocks have been used, *viz.* *y w*, f^5, *w sn*, *y* f^5 *car*, *y w sn*, and wild type stock. The different combinations of the stocks are clear from Table 1, where the series are arranged in chronological order.

Series *a* showed a very low frequency of gynandromorphs and therefore the dose was doubled in the following series (*b*—*m*).

The stocks in series *a* and *b* did not contain a sufficient number of marker genes and therefore the results were not quite reliable. By using stocks with more marker genes this has been avoided on the whole in the latter series.

The frequencies of eliminations of the treated X-chromosomes in series *c*, *d* and *e* showed such great differences that four new series were started, *viz.* series *h*, *j*, *k* and *l*, with common treatment to find out whether there were real differences or errors in the former experiments. Immediately after treatment the flies were divided into respective groups.

In series *a* and *b* the age of the parental flies was not determined.

In the following series, *c*—*m*, the age of males was 0,5 ± 0,5 days and that of females 1—3 days, if there is no other statement in Table 1.

For comparison the control series from the X-ray experiments (BONNIER and LÜNING, 1951, Table 1) are included in Table 1 as series *n*.

TABLE 1. *Frequencies of gynandromorphs. The treated parents are in italics or, when wild type, underlined. When one of the parents is of wild type the table contains only gynandromorphs due to eliminations of the treated X-chromosome.*

Series	Parents	Age at		Tolal females and gyn.	Gynandromorphs due to					
					maternal eliminations			paternal eliminations		
		treating ± 0,5 days	mating ± 0,5 days		1	2	1+2 per 10^4	1	2	1+2 per 10^4
a	*y w* × *f*^{5}	—	—	62701	16	—	2,55	15	—	2,39
b	*y w* × f^5	—	—	13528	6	—	4,44	2	—	1,48
c	*w sn* × y f^5 car	0,5	0,5	37951	18	2	5,27	4	—	1,05
d	y w sn × ±	0,5	0,5	39281				35	7	10,69
e	± × y w sn	0,5	0,5	11735	19	3	18,75			
f	y w sn × ±	0,5	7,5	7150				5	3	11,19
g	y ws n × ±	7,5	7,5	10625				6	7	12,24
h	*w sn* × y f^5 car	7,5	7,5	27413	15	—	5,47	2	—	0,73
j	± × y w sn	7,5	7,5	24095	27	8	14,53			
k	y w sn × ±	0,5	0,5	19628				22	3	12,74
l	y w sn × ±	0,5	7,5	19692				16	7	11,68
m	*y f*^{5} *car* × w sn	0,5	0,5	20562	22	1	11,19	5	—	2,43
n	w sn × y f^5 car	—	—	221405	22	11	1,49	18	5	1,04

DISCUSSION.

I. *Comparisons between series in Table 1.* — In series *b*, *c*, *h*, and *m*, where female were treated, and in the control, series *n*, both parental X-chromosomes contain marker genes. Hence, one can distinguish between eliminations of maternal and paternal X-chromosomes. From Table 1 it is clear that on comparison between the control *versus* the treated series *b*, *c*, *h* and *m* the frequency of eliminations of maternal, treated X-chromosomes is more or less increased, whereas the frequency of eliminations of paternal, untreated X-chromosomes is not increased. This result is different from that after X-ray irradiation of females where the frequency of eliminations of maternal, treated X-chromosomes is

not increased, while the frequency of eliminations of paternal, untreated X-chromosomes is increased in one group of series (BONNIER and LÜNING, 1951).

That the frequency of eliminations of paternal, untreated X-chromosomes is not increased indicates that the post-treatment with compressed air has freed the flies from the mustard gas atmosphere and that there seems to be no secondary influence.

The frequency of eliminations of maternal, treated X-chromosomes is not the same at treatment of females of different stocks. This is clear from a comparison between series *c* and *h* partly *versus* series *e* and *j* and partly *versus* series *m*. As indicated above, series *h* and *j* were treated together and therefore they could be compared directly. A χ^2-test gives $\chi^2 = 22$ and $P < 0{,}001$. This difference will be dealt with below.

In order to ascertain whether there are any differences on common

TABLE 2. *Frequencies of gynandromorphs and mutations with regard to length of time from mating to egg laying. I = 1—5 days, II = 6—10 days, and III = 11—14 days from mating to egg laying.*

Series		I	II	III
h	Total number	4958	13294	9161
	Gyn.: abs. number	3	5	7
	per 10^4	6,05	3,76	7,64
j	Total number	3319	10166	10610
	Gyn.: abs. number	15	4	16
	per 10^4	45,2	3,93	15,1
	Mut.: abs. number	2	5	2
	per 10^4	6,03	4,92	1,89
k	Total number	6568	8350	4710
	Gyn.: abs. number	12	9	4
	per 10^4	18,3	10,8	8,49
	Mut.: abs. number	6	3	8
	per 10^4	9,14	3,59	17,0
l	Total number	7386	7588	4718
	Gyn.: abs. number	10	7	6
	per 10^4	13,5	9,23	12,7
	Mut.: abs. number	4	6	5
	per 10^4	5,42	7,91	10,6
m	Total number	4797	9271	6494
	Gyn.: abs. number	14	6	3
	per 10^4	29,2	6,47	4,62

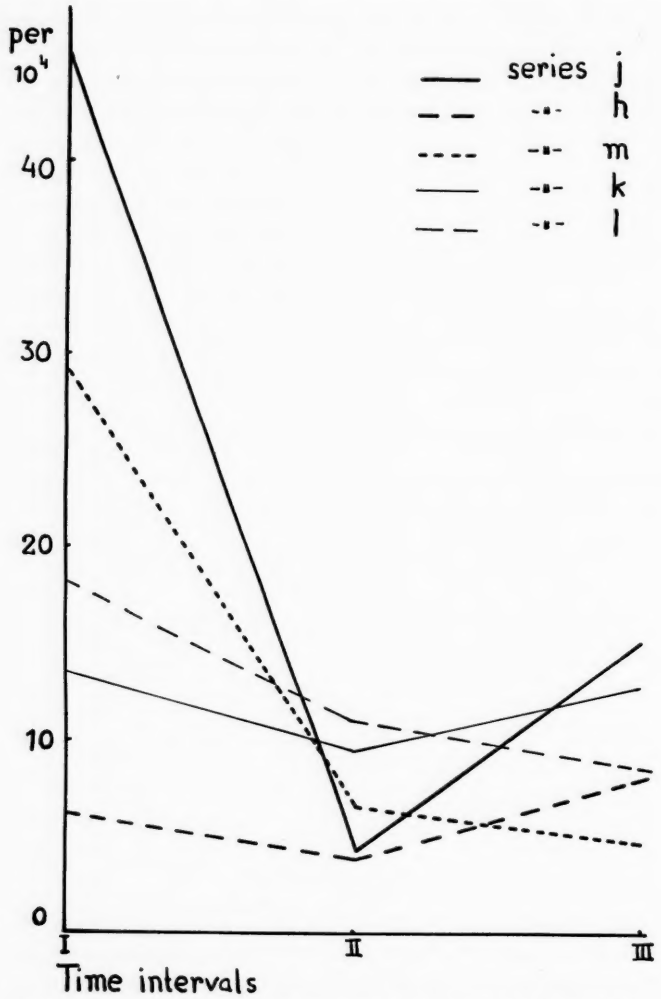

Fig. 1. Frequencies of gynandromorphs in the three time intervals.

treatment of females and males of the same stock, series *j* will be compared to series *k* and *l*. They do not show any significant differences. This result too is different from that after X-ray irradiation.

In order to find out, whether age has any influence on treatment of females, females of both *w sn* and wild type stock were treated for

$0{,}5 \pm 0{,}5$ days and $7{,}5 \pm 0{,}5$ days. See series *c*, *e* and *h*, *j*. No differences were observed.

The influence of age of males has also been examined. Males of wild

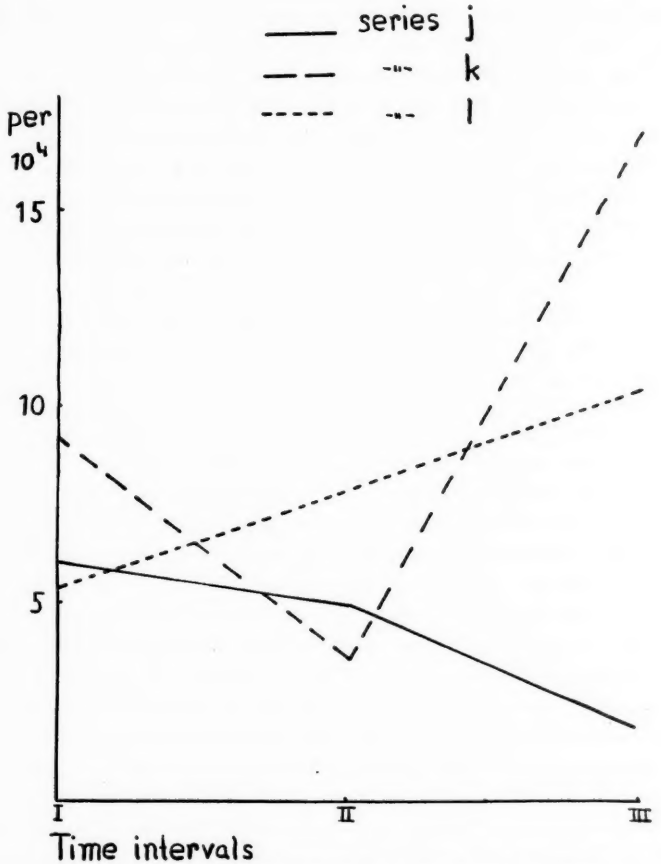

Fig. 2. Frequencies of mutations in the three time intervals.

type stock were treated partly at $0{,}5 \pm 0{,}5$ days and then either mated immediately, series *d* and *k*, or kept without females 7 days before mating, series *f* and *l*, and partly at $7{,}5 \pm 0{,}5$ days and mated immediately, series *g*. The frequencies of eliminations of paternal, treated X-chromosomes do not show any significant differences. Hence, the age

seems not to have any influence. This too is different from that after X-ray irradiation (BONNIER and LÜNING, 1951).

By letting the females lay their eggs on agar plates, which were changed at 24 hours' intervals it was possible to classify all aberrations in days after treatment. Owing to the frequencies of eliminations being too low to be divided into days they are grouped in the following manner: I = 1—5 days, II = 6—10 days, and III = 11—14 days. Series *h*, *j*, *k*, *l*, and *m* were subdivided into these three groups, see Table 2 and Fig. 1. χ^2-tests of the three groups were made. Only in the first group significant differences exist between the series, $P < 0{,}001$. In the two other groups P is about 0,20. These differences will be dealt with below.

The frequency of mutations in the three loci *y*, *w* and *sn* at treatment of females and males of wild type stock is included in Table 2 and Fig. 2. The total number of mutations is too low to make a statement about possible differences. It seems, however, as if the frequency of mutations was somewhat higher at treatment of males than at treatment of females.

II. *The causes of production of gynandromorphs.* — In the introduction the power of X-rays to induce gynandromorphs was mentioned. In order to explain those processes which then take place there are in the main two hypotheses. First PATTERSON and STONE's (1938) which says that at treatment of males the chromosomes of the sperm are already split into their chromatids and that it may happen that at treatment only one of the chromatids is affected. The other hypothesis, based on hypotheses of MULLER (1940) and PONTECORVO (1942), is described in detail by BONNIER, LÜNING and PERJE (1949). According to this, the breaks occur before splitting into chromatids, and at formation of gynandromorphs one of the chromatids is supposed to restitute and the other remains broken and is eliminated for that reason.

The question is then whether treatment with sulphur mustard gas can induce gynandromorphs due to breaks and, whether in that case, breaks are the only cause or there are other causes.

The first question was whether mustard gas can induce breaks which lead to formation of gynandromorphs. In Table 1 the series *d*, *f*, *g*, *k*, and *l* with treated wild type males mated to *y w sn* females include 111 gynandromorphs, where the treated, paternal, X-chromosome was eliminated. Among these, there are five cases which in their male area showed y^+ *sn* or, if the eyes were included, y^+ *w sn*. Moreover, there is one case which showed three phenotypically different areas, *viz.* +, *y*, and *y w sn*, the latter including secondary male sexual characters.

These six cases can only be explained by the supposition that at least two breaks had occurred in each case. By that it is proved that breaks induced by mustard gas can lead to formation of gynandromorphs.

In those series (*e* and *j*), where wild type females were treated and mated to *y w sn* males, there is among 57 gynandromorphs, due to elimination of the treated, maternal, X-chromosome, no case in which the male area showed y^+ *sn* or y^+ *w sn*. Hence, there is no proof that breaks really may cause eliminations when treating females, though there is reason to suppose that this is the case.

If from Table 1 all series with treated females and males of wild stock are totalled the number examined F_1 daughters is 132206 of which 168 are gynandromorphs or 12,71 per 10^4. The frequency of mutations in the three loci *y*, *w* and *sn* calculated for the three series *j*, *k* and *l* of Table 2 is 6,47 per 10^4. At complete independence between mutations and breaks and with the given number of F_1 daughters one should expect to find $12{,}71 \cdot 6{,}47 \cdot 10^{-8} \cdot 132206 = 0{,}11$ gynandromorphs, which in the female area should show a mutation in *y*, *w* or *sn* locus. Two cases of that type have been found. One, after treated females mated to *y w sn* males, which in its female area was *y* and in the male *y w sn*. The other case, after treated males mated to *y w sn* females, showed in its female area *sn* and in its male area *y sn*. A χ^2-test gives $\chi^2 = 32$, which corresponds to a P which is considerably below 0,001. The χ^2-test exaggerates, however, at such low frequencies the discrepancy between observation and expectation. In regard, however, to the very small magnitude found for P it may reasonably be supposed that the test is significant. This indicates that mutation and elimination in these cases are probably not independent. This can be explained in different ways: (1 a) The chromosome is broken before splitting; one chromatid is restituted producing a mutant allele and the other chromatid remains broken and is thus eliminated. — (1 b) The primary effect is creation of an instability which after splitting produces a mutant allele in one of the chromatids and a break in the other. — (2) The chromosome is split into chromatids and then affected. In that case the same mutagenic factor must cause a break in one of the chromatids and a mutation in the other in the same locus or in the vicinity.

It was indicated above that there are significant differences in the frequencies of eliminations between different stocks. This may possibly be due to other causes than breaks. The reason for this assumption is clear from the following. By comparison between the series in Table 1 it was shown that there is a significant difference between the fre-

quencies of gynandromorphs after treatment of *w sn* and of wild type females. In Table 2 the frequencies of the three time intervals are calculated for five series and graphically shown in Fig. 1. From this it is clear that there do not exist any differences between the three intervals after treatment of *w sn* females, whereas treated wild type females show a very high frequency of gynandromorphs during the first time interval while the two later intervals show about the same frequencies as the treated *w sn* females. The high frequency of gynandromorphs during the first interval of the treated wild type females may be explained either by supposing that this stage is especially susceptible and thereby received a greater dose or that in this stage there are also other processes than breaks which lead to formation of gynandromorphs. Against the former assumption speaks the fact that the frequency of mutations during the first interval did not show a corresponding pike; see Table 2 and Fig. 2. Thus the latter assumption seems to be the best founded. The processes which lead to eliminations may be centromere- or plasma-effects. Further experiments are under preparation.

III. *Formation of gynandromorphs.* — It was shown above that a cause for formation of gynandromorphs is the origin of breaks. So the task is to determine when a break, which leads to gynandromorphy, is originated. At X-ray irradiation it is supposed that breaks are induced immediately. That the same is the case at treatment with mustard gas is not established but is discussed below.

In order to compare the effect of mustard gas at treatment of the two sexes one must be acquainted with the gametogenesis in the two sexes. In freshly hatched males there are mature spermatozoa in such a large number that it takes 12—19 days for their exhaustion (DEMEREC and KAUFMANN, 1941; LÜNING, unpublished). In females HUETTNER (1924) has proved that the oöcytes remain in metaphase I till fertilization, when the meiosis is completed. Consequently, at treatment of males the mature gametes are exposed but at treatment of females it is the oöcytes I or oögonia that are exposed. So the chromosomes in the oöcytes and oögonia have to pass through at least one division before the gamete-stage is reached. For that reason one may suppose that, if a break is induced in an X-chromatid in oöcyte I and the broken X-chromosome is included in the egg nucleus, the distal part of the broken X-chromosome is lost in meiosis II and that the proximal part of the broken X-chromosome is eliminated during the first cleavage division of the zygote by formation of a fusion bridge and resulting in an XO male or a YO zygote; the latter dies in an early embryonic stage. Thus with

the assumption above one would not expect any increase of the frequency of gynandromorphs at treatment of females.

As was stated above, X-ray irradiation has an immediate effect and at treatment of females with X-rays one would thus not expect any increase of the frequency of the gynandromorphs. This is in agreement with the results of BONNIER and LÜNING (1951).

At treatment of females with mustard gas a more or less strong increase of the frequency of gynandromorphs is proved, according to Table 1. From this one can conclude that there is not an immediate formation of breaks. Instead, they are formed at a later stage, *viz.* after meiosis II, in the haploid egg nucleus. The cause of this delay may be explained in one of the following ways. Either the mutagenic factor is only effective under certain conditions — *e. g.* haploid stage, or it may in a comparatively short time after treatment affect the chromosomes in such a manner that the final effect — the break — is not formed before one or more chromosome divisions are passed through.

If the first of these suppositions be true, then the mutagenic factor must affect selectively the maternal X-chromosome during the short time the haploid egg nucleus exists and the sperm-nucleus approaches or lies close to the egg nucleus. This does not seem probable.

The second supposition, where the mutagenic factor in a comparatively short time after treatment should cause a state in the chromosome that in the haploid stage or even later would result in a break, is supported by the observations of AUERBACH (1947) who found four cases, after treatment of males, where the final effect was not formed until the next generation. This was explained by the supposition that the primary effect consisted in creation of an instability and that this is stabilized at a later stage. Thus this second supposition seems to give the best explanation of the occurrence of an increased frequency of gynandromorphs when treating females.

It was said above that the earliest stage in which breaks are able to form gynandromorphs is the gamete stage. Breaks at later stages may also lead to gynandromorphs. There is, however, reason to suppose that most breaks occur at the gamete stage. This is clear from the following discussion. PATTERSON and STONE (1938) have shown that the proportion between female and male areas is 2 : 1 in X-ray induced as well as in spontaneous gynandromorphs. BONNIER, LÜNING and PERJE (1949) have shown that the proportion between female and male areas is irrelevant for the study of when an X-chromosome elimination occurs. The fact that female areas on the average are twice as large as male

areas is thought not to necessitate the assumption that a number of eliminations are initiated in later cleavage nuclei. All gynandromorphs in this experiment have been depicted. The drawings have been used for a comparison between the distribution of female and male areas. Gynandromorphs from treated females as well as treated males showed the same proportion, 2 female parts to 1 male part, or the same proportion as after X-ray treatment of males. So there is reason for the assumption that at least the majority of the breaks which lead to formation of gynandromorphs are induced before the beginning of the second cleavage division and probably before formation of the zygote.

From the above discussion it is clear that at mustard gas treatment of males there is reason to suppose that in the majority of cases there is no delay in break formation comparable with that after treating females.

IV. *Indirect effects?* — It was shown above that when treating females there was no increase in the rate of gynandromorphs due to elimination of the untreated, paternal, X-chromosome. The plausible conclusion is that there is no secondary effect. In the series *b*, *c* and *h* in Table 1 there are, however, 5 *w*-mutations in a total of 78892 F_1 daughters. In the control, series *n*, there is in 221405 animals only 1 *w*-mutation (fractional). These data indicate that perhaps there is an indirect influence on the untreated X-chromosome.

Acknowledgements. — The author is deeply indebted to Professor GERT BONNIER for his many valuable suggestions. I wish also to thank Dr. H. BOREI for help in providing sulphur mustard gas; Dr. A.-M. PERJE, Mrs. S. HENNINGS, Mrs. B. ARNBERG, Miss G. VON ESSEN, Miss T. RIBBING, and Mr. J. HAMMARÉN for all help in counting and classifying the flies.

The present investigation was supported by the Swedish Natural Science Research Council.

SUMMARY.

(1) Males and females of different stocks of *Drosophila melanogaster* have been treated with sulphur mustard gas and F_1 daughters analysed as regards gynandromorphs.

(2) The frequency of gynandromorphs due to elimination of the treated X-chromosome is more or less increased as compared with the controls.

(3) When treating females the frequency of gynandromorphs due to elimination of the untreated, paternal, X-chromosome was about the same as in the controls.

(4) Females and males of wild type stock were treated together and then mated to *y w sn* animals. Both sexes gave the same frequency of gynandromorphs due to elimination of the treated X-chromosome.

(5) Females and males of different ages were treated. There was no aging effect.

(6) Breaks are supposed to be the cause of eliminations after treatment of males. It has not been possible directly to prove the same for females.

(7) Two cases were found in which a gynandromorph showed a mutant character in the female area. Break and mutation seem in these cases not to be independent.

(8) The time between treatment and laying of eggs, from which aberrations arise is checked by a special method. The aberrations are then grouped in three time intervals.

(9) The division of the gynandromorphs into the three time intervals show that when treating + and *y* f^5 *car* females there is a very high frequency of gynandromorphs in the first interval. A possible explanation is that there are also other processes than breaks that produce gynandromorphs, *e. g.* centromere- or plasma-effects.

(10) There is reason to suppose that the majority of breaks occur in the gamete stage, whether females or males are treated.

(11) The increase of the frequency of gynandromorphs when treating females may be explained by supposing that there is primarily a creation of an instability and that it is stabilized in most cases in the gamete stage.

(12) After treatment of *y w*, and *w sn* females 5 *w*-mutations in the untreated, paternal, X-chromosome have been found.

LITERATURE CITED.

1. AUERBACH, C. 1943. Chemically induced mutations and rearrangements. — Drosophila Information Service (DIS) XVII, 48—50.
2. — 1947. The induction by mustard gas of chromosomal instabilities in *Drosophila melanogaster*. — Proc. of the Royal Society of Edinburgh, LXII, 307—320.
3. BONNIER, G. and LÜNING, K. G. 1951. Spontaneous and x-ray induced gynandromorphs in *Drosophila melanogaster*. — Hereditas XXXVII, 469—487.

4. BONNIER, G., LÜNING, K. G. and PERJE, A.-M. 1949. Studies on X-ray mutations in white and forked loci of *Drosophila melanogaster*. II. A study of the formation of gynandromorphs and other kinds of mosaics. — Hereditas XXXV, 301—336.
5. DEMEREC, M. and KAUFMANN, B. P. 1941. Time required for *Drosophila* males to exhaust the supply of mature sperm. — Amer. Nat. LXXX, 366—379.
6. HUETTNER, A. F. 1924. Maturation and fertilization in *Drosophila melanogaster*. — Journ. Morph. 39, 249—265.
7. MULLER, H. J. 1940. An analysis of the process of structural change in chromosomes of *Drosophila*. — Journ. Genet. 40, 1—66.
8. PATTERSON, J. T. 1931. The production of gynandromorphs in *Drosophila melanogaster* by X-rays. — Journ. Exp. Zool. 60, 173—211.
9. PATTERSON, J. T. and STONE, W. 1938. Gynandromorphs in *Drosophila melanogaster*. — The Univ. of Texas Publ. 3825, 1—67.
10. PONTECORVO, G. 1942. The problem of dominant lethals. — Journ. Genet. 43, 295—300.

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ON THE FORMATION OF REDUCED AND UNREDUCED GAMETOPHYTES IN THE FEMALES OF ANTENNARIA CARPATICA

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I. INTRODUCTION.

IN 1935 the author published a paper on the cytology of the Scandinavian *Antennaria* species. Among these *A. carpatica* was also included, but concerning this species I could only give the somatic chromosome number which was established as $2n = 40—42$. A close investigation of the micro- and macro-sporogenesis could not be made, since the collected buds were too old. However, it was possible to make some observations regarding the mature pollen grains and ESs. Thus the pollen grains were irregular in size, dwarf grains especially were numerous. The mature ESs never produced embryos but degenerated gradually and were dissolved. It was evident, therefore, that this plant merited a more careful investigation, and such a one I have now carried out; the results are presented in this paper.

A. carpatica is a dioecious plant rarely encountered in the northern part of the Scandinavian mountains. Consequently, it is no easy matter to get suitable material of this plant, yet I have received it from three different localities in Sweden: from Peltsa in the most northern part of Sweden (from Dr. A. NYGREN, Uppsala), from Pite Lappmark (from Mr. P. O. NYMAN, Uppsala), and from Torne Lappmark where I collected it myself in the environments of Abisko. Unfortunately, the material is complete only with respect to the female plants. As regards the male plants, some important stages are still missing, but I hope to be able to collect these in the future. The present paper, therefore, will deal mainly with the females and only a few particulars will be given on the males. The cytology of the latter will be discussed fully when I have completed the material.

In the following we will on several occasions deal with apomixis problems. As to the terminology, I follow the scheme of FAGERLIND (1940) which seems to me to be the most appropriate and complete of those proposed in recent years.

II. THE FORMATION OF THE EMBRYO SAC (ES).

In this plant the ES can be formed in at least three different ways. One of these gives ± reduced ESs while the other two give unreduced ESs. I shall begin with an account of the former type.

1. THE FORMATION OF ± REDUCED EMBRYO SACS.

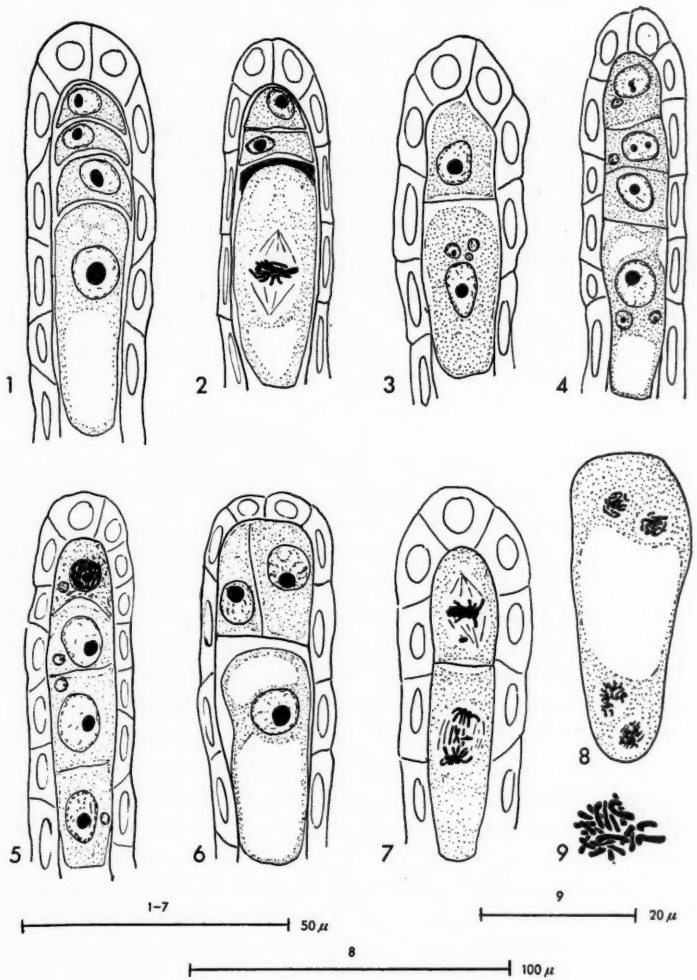
The youngest stages in the development of the female gametophyte reminds one very much of the conditions in *A. dioica* (*cf.* JUEL, 1900). Thus, the nucellus contains only one archesporial cell which divides to give four daughter cells, of which the basal one forms the ES, as indicated in Figs. 1—2. Closer studies of the dyad and tetrad stages, however, disclose remarkable deviations. Often these stages look like those in Figs. 3—5. In Fig. 3, one dyad is to be seen with three micronuclei in the basal cell. Figs. 4 and 5 show two different tetrads, each with several micro-nuclei. In Fig. 6, a much stronger deviation appears; a triad has been formed the basal cell of which looks as if it was going to form the ES.

A sporogenesis of this type indicates that the meiosis must be irregular. In the material hitherto at my disposal, however, only two ovules have appeared with meiotic stages. One of these is shown in Fig. 7. It is a dyad with the nuclei in division. As may be seen from the figure, the two divisions are irregular, especially the lower one with several laggards. In the second ovule, the meiosis is also irregular but this will be discussed in the next chapter.

These irregularities in the macro-sporogenesis are in excellent agreement with the observations I made in 1935 concerning the microsporogenesis and which are mentioned in the introduction to the present paper. Thus, dwarf pollen grains were common in the loculi and after a study of the new material I have been able to confirm that the size of the pollen grains is very variable. Dwarf grains are especially common. In a few loculi meiosis could be studied but, unfortunately, rather poor fixation made a closer analysis impossible. It was obvious, however, that the division was very irregular with several univalents and polyvalents.

In most ovules examined in this plant, sporogenesis takes place in the above-mentioned manner resulting in ± reduced ESs. Some of them die in different stages of development, often in the tetrad stage. The most vital ones are probably those which have got a chromosome number as close as possible to the haploid number. Fig. 8 shows a four-nucleate

ES with the nuclei in division. One of the divisions is drawn in Fig. 9 and about twenty chromosomes are to be counted.



Figs. 1—9. *Antennaria carpatica.* — 1, normal tetrad. — 2, normal tetrad, the basal megaspore in division forming the ES. — 3, dyad with micro-nuclei. — 4—5, tetrads with micro-nuclei. — 6, triad. — 7, dyad, the second meiotic division is irregular. — 8, four-nucleate ES in division. — 9, one of the divisions in Fig. 8 in higher magnification; about 20 chromosomes are to be counted.

2. THE FORMATION OF UNREDUCED EMBRYO SACS.

Unreduced ESs can be formed in *A carpatica* in two different ways: (1) through restitution nuclei, (2) through somatic apospory.

A. EMBRYO SAC FORMATION THROUGH RESTITUTION NUCLEI.

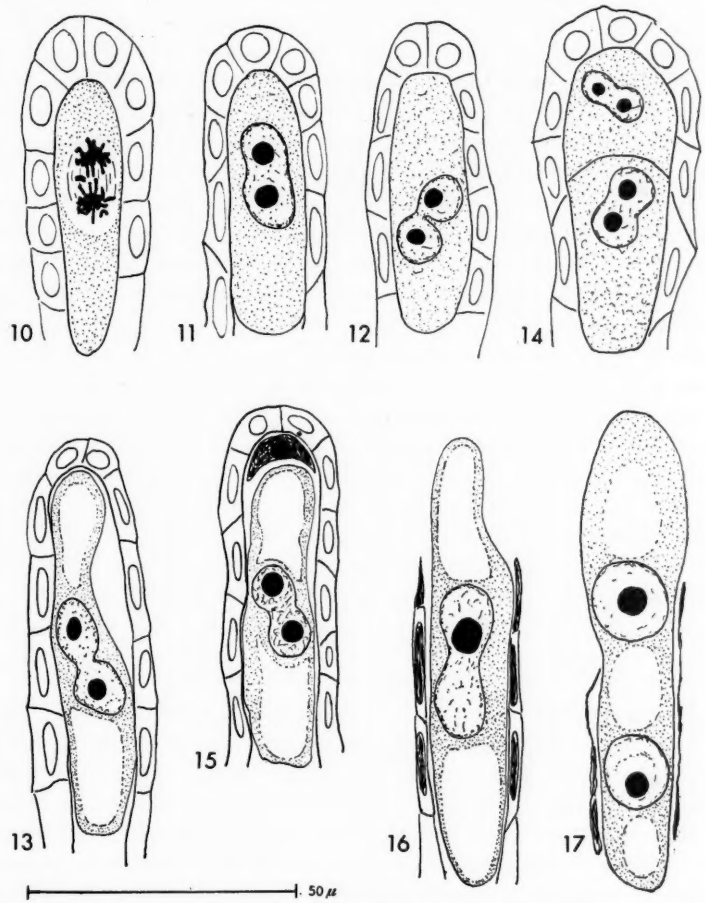
In studying the young ovules, one finds remarkably often cases such as indicated in Figs. 11—15. On account of their shape and appearance, the nuclei in these figures must in all probability be restitution nuclei. In Figs. 11—13 such a nucleus is formed in the first meiotic division, in Figs. 14—15 in the second. The qualifications for a restitution nucleus, *i. e.* a disturbed meiosis, are also present, as has been mentioned earlier. An examination of the *micro-sporogenesis*, however, reveals no restitution nuclei at all nor have dyads been observed at a stage corresponding to the finished tetrads. Instead, the loculi have been filled with mere polyads. Hence, it follows that *there must be a factor favouring the formation of restitution nuclei in the macro-sporogenesis of A. carpatica, a factor, however, which does not affect the micro-sporogenesis.*

This is strongly reminiscent of the conditions of some agamosperms as, for instance, *Taraxacum*, *Erigeron*, and *Chondrilla* (*cf.* FAGERLIND, 1947 a and b; BERGMAN, 1950). In these plants, restitution nuclei can be present in nearly all EMCs but are relatively scarce in the PMCs. These agamosperms also have an irregular meiosis but it is not this alone which causes the restitution nuclei in their ovules. Rather, these nuclei are caused by the fact that the first meiotic division stops at some stage during prophase—anaphase and then changes directly into telophase, often with a marked contraction of the chromatin mass.

At present I cannot state definitely whether the restitution nucleus in *A. carpatica* is formed in the same way but it is certain that there must be something remarkable in the meiosis in certain ovules of this plant, a fact which is supported by Fig. 10. This figure shows the first meiotic division at late anaphase; apparently the chromosomes are very much elongated, almost mitotically (*cf.* Fig. 2). That it must be a meiosis, however, is indicated partly by the laggard at the metaphase plane and partly because the number of chromosomes at each pole is estimately the same as the reduced number.

It is not easy to decide how this division should be interpreted, especially since this one and that shown in Fig. 7 are the only meioses I have encountered in this material. The meiosis in Fig. 10 reminds the author, however, of the metaphases—anaphases with sticky chromo-

somes which I found in the agamospermous *Erigeron annuus* and which are shown in Fig. 1 e—g (BERGMAN 1944, p. 250). In that paper I suggested (p. 250) that these sticky metaphases—anaphases could give



Figs. 10—17. *Antennaria carpatica.* — 10, EMC with a very irregular meiosis. — 11—15, different types of restitution nuclei. — 16, unreduced one-nucleate ES. — 17, unreduced bi-nucleate ES.

rise to those restitution nuclei which are also to be found in *Erigeron annuus* (BERGMAN, 1944, Fig. 1 h—i), a statement which I was able to support in my subsequent studies of similar divisions in *Chondrilla*

juncea (BERGMAN, 1950, pp. 316—317). In my opinion these sticky metaphases—anaphases have been formed in such a way that the division has stopped for some reason or other and the stickiness is nothing but telophasical changes of the chromosomes. The anaphase, shown in Fig. 10 in the present paper, also appears sticky and can possibly be interpreted in a corresponding manner. It is difficult to say if the restitution nuclei in the second meiotical division are formed in a similar way. The anaphase in Fig. 7 with its elongated and strangely orientated laggards also looks peculiar. Further studies, however, are necessary before these questions can be decided.

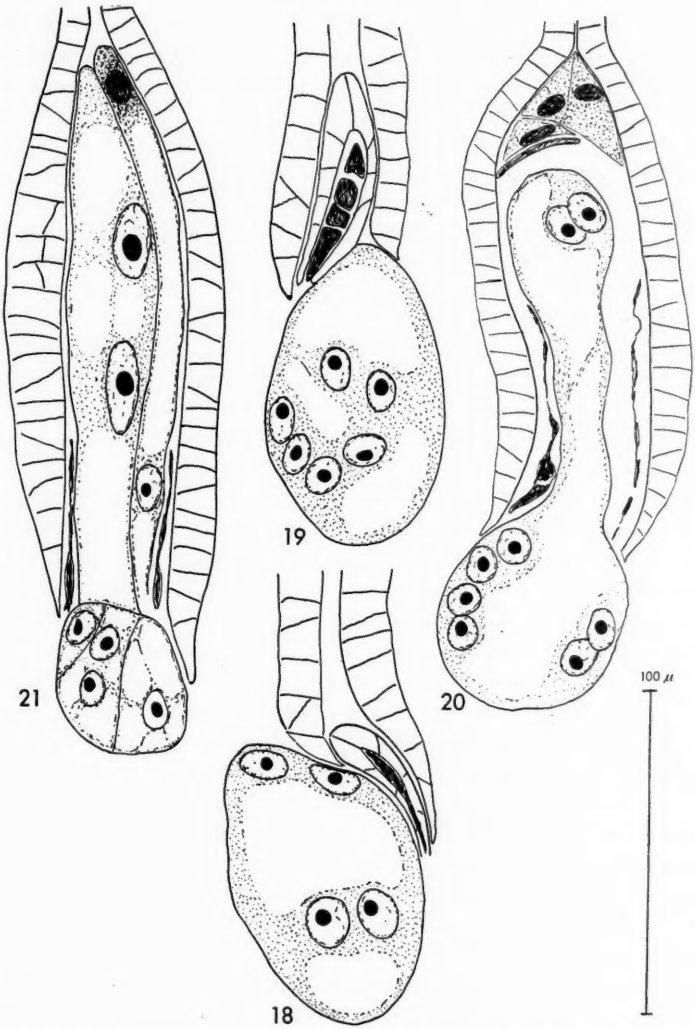
In Figs. 11—12, the restitution nuclei must be relatively young while in Figs. 13 and 15, quite a long time must have passed since they were formed, as is evident from the strong vacuolization of the cytoplasm. It is remarkable that the nuclei are not rounded at this stage but still retain their dumb-bell shape. Similar cases, however, have been observed by the author in the micro-sporogenesis of the dyad forming plants of *Chondrilla juncea* (*cf.*, *e. g.*, Fig. 64, BERGMAN, 1950).

It is apparent from Figs. 16—17 that the restitution nuclei are able to form vital ESs. Fig. 16 shows a one-nucleate ES which, having recently pierced the nucellar epidermis, is pushing forward in the direction of the micropyle. Fig. 17 may be interpreted as a later stage where the nucleus has divided and given rise to a bi-nucleate ES with very large nuclei. Consequently, among the mature ESs, one can expect that several are unreduced, being formed in this manner through restitution nuclei.

B. EMBRYO SAC FORMATION THROUGH SOMATIC APOSPORY.

Unreduced ESs can also be formed through somatic apospory in *A. carpatica*. In the relatively limited material of buds at my disposal I have come across some 20 cases, some of which have been drawn in Figs. 18—24. The aposporous ESs of this plant originate and develop in the same manner as in *Hieracium* subgen. *Pilosella* (ROSENBERG, 1927) or *Leontodon hispidus* (BERGMAN, 1935 b). Thus, they are generally formed from a chalaza cell in close proximity to the nucellus, as shown in Figs. 18—19. In these two cases, the aposporous ESs have strongly developed and reached the four- and six-nucleate stage, respectively. In both cases, expansion of the ESs has crushed the nucellus to one side with the result that its contents have degenerated. In Fig. 20 we have a somewhat later stage showing approximately the same development. Here an aposporous ES has pushed its way from the chalaza

into the ES-cavity where a sporogenous ES has already developed. The latter, however, has become greatly compressed by the aposporous one and apparently is about to degenerate.



Figs. 18—21. *Antennaria carpatica.* — Somatic apospory in different stages of development.

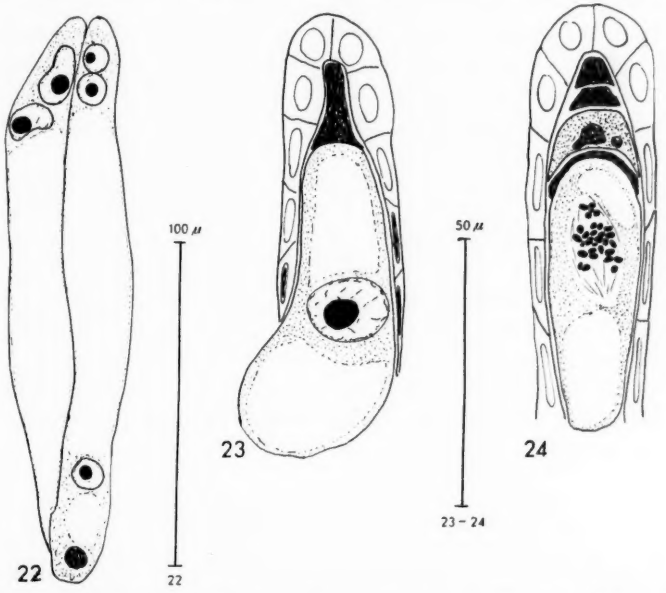
Fig. 21 represents a somewhat different case. Here, out of a complex of aposporous cells situated in the chalaza, only one has penetrated into the ES-cavity and is competing with the sporogenous one for space. Both cells are bi-nucleate, but as the micropylar nucleus of the sporogenous ES shows obvious signs of degeneration, the aposporous one had probably formed the definitive ES.

This competition for space between the aposporous and sporogenous ES has produced in several ovules very complicated figures. Only on rare occasions does one come across such relatively uncomplicated cases as indicated in Fig. 22, where a bi-nucleate aposporous and a four-nucleate sporogenous ES lie side by side in the ES-cavity.

In *Hieracium excellens*, ROSENBERG (1907; *cf.* Figs. 25—26) found that the aposporous ES often originated from a somatic cell immediately under the EMC or its daughter-cells and at an early stage had pushed them aside and replaced them in the nucellus. This also occurs in *Antennaria carpatica* which appears from Fig. 23. That this really is a case of an aposporous ES is apparent, partly from its position and partly from the size of the nucleus. The size of the nucleus is obvious if one compares the reduced nucleus in Fig. 1 with the unreduced ones in Figs. 16—17.

Probably a corresponding case is represented in Fig. 24 where an aposporous ES has penetrated into the nucellus, crushing a tetrad whose earlier appearance corresponded approximately to that in Fig. 5. In this figure, the nucleus of the aposporous ES is in division, the latter being rather peculiar. The chromosomes are scattered over the spindle and are strongly, nearly spherically, contracted. Their number is about 40 but all are not drawn. Another interpretation of this figure might be that of an ovule which at an earlier stage possessed two legitimate EMCs in the same nucellus, the basal one of which later developed at the expense of the other. Against this interpretation, however, it may be pointed out that a normal EMC does not divide after the cell plasm has begun to vacuolize in the manner apparent in the figure. More likely it is a question of a somatic cell, the division of which has been meiotisized for some reason or other. True, the ability of chalazal cells to show meiotical tendencies and even in some cases carry through a meiotic division is very rare; nevertheless it is known in some plants. For example, it has been demonstrated in two different individuals of *Leontodon hispidus* (BERGMAN, 1935 b, Figs. 42, 57, 59 and 60), and the case which BHADURI, according to MAHESHWARI (1950, Fig. 50 D), found in *Solanum melongena* must also belong to this category.

In *Galium* there is a case of special interest for the interpreting of the division in *A. carpatica* in question. In the ovules of *Galium Mollugo* (*cf.* also *Crucianella*), FAGERLIND (1937, p. 222, Figs. 5—6 and 1940, pp. 43—44) came across divisions of a type which could be characterized as something between mitosis and meiosis. *Galium Mollugo* has a multicellular archesporium, the boundary between archesporium and somatic tissue being very diffuse. In the periphery of the archesporium



Figs. 22—24. *Antennaria carpatica.* — 22, one aposporous ES to the left and one sporogenous to the right, side by side in the same ovule. — 23, one aposporous ES from the chalaza replacing the sporogenous in the nucellus. — 24, one aposporous ES has pushed its way into the nucellus crushing a tetrad. The first division of the ES is meiotisized, forming a semiheterotypic division.

there occur cells which according to their appearance can be regarded neither as somatic nor as archesporial cells. Instead they form a transition between these two cell types. In the centre of the archesporium the cells divide meiotically with normal bivalent formation, but as we move toward the periphery of the archesporium, the chromosome associations become looser until finally at the boundary or outside of the archesporium the divisions are completely asynaptic. Everywhere the chromosomes have the same contracted shape as in the first division of a

normal meiosis. The ± asynaptic divisions are carried through as semiheterotypical.

In a later paper on *Elatostemma*, FAGERLIND (1944, p. 101) declares concerning these conditions in the macro-archesporium of *Galium Mollugo*: »Die Verhältnisse dort lassen sich ausgezeichnet erklären mit Hilfe der Annahme, dass in dem Archespor eine Meiose hervorrufende Substanz vorliegt, deren Konzentration mit der Zeit und sukzessiv in den mehr peripher gelegenen Partien abnimmt». The division shown in Fig. 24 of the present paper reminds one very much of the meiotisized divisions of the somatic cells in the periphery of the archesporium in *Galium Mollugo* (*cf.*, *e. g.*, Figs. 5—6 in FAGERLIND, 1937) and could be interpreted in a corresponding manner. The cell in question is situated in the nucellus where the meiosis hormones ought to occur, but on account of the lateness of the division, in a less concentrated state. Therefore they have not been able to cause a real meiosis but only a semiheterotypic one. Other workers have interpreted similar meiotisized divisions in somatic cells with the aid of hormone theories (*e. g.* ISHIKAWA, 1911; GUSTAFSSON, 1939; STEBBINS, 1942). It must, however, be pointed out that so far no one has proved that such hormones actually exist.

Whether the other somatically aposporous ESs of *A. carpatica* are also formed through meiotisized divisions I cannot say, Fig. 24 being the only division I have discovered.

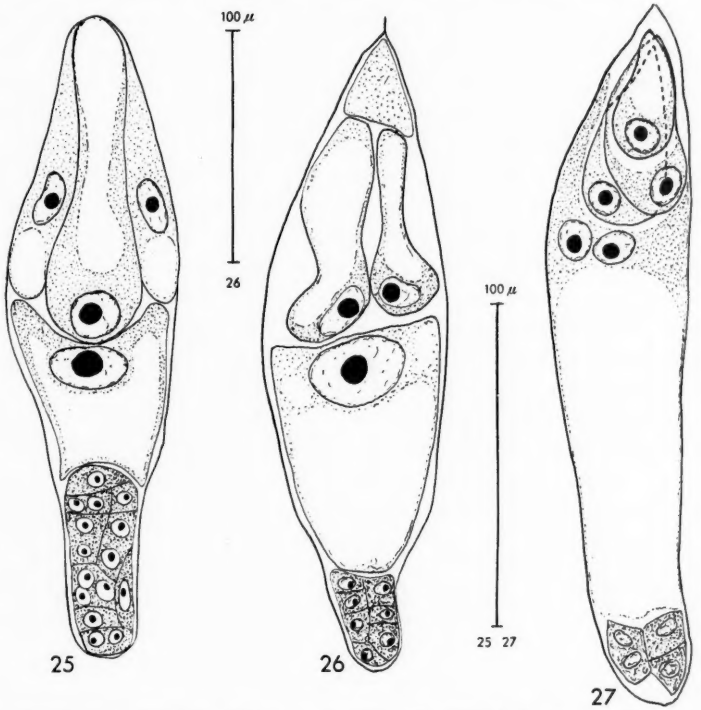
III. THE MATURE EMBRYO SAC.

In spite of the abnormal meiosis mentioned above one encounters a remarkably number of ESs which have developed normally. Probably this is due to several ESs being unreduced and formed through restitution nuclei or somatic apospory. Fig. 25 illustrates a normally organized ES of the type common in the genus *Antennaria*. Especially characteristic is the antipodal apparatus with its numerous deeply coloured cells (*cf.* JUEL, 1900). Frequently, however, one comes across ESs either with abnormal organization or lacking organization altogether. Sometimes they have such a complicated appearance that it is impossible to draw them. I have selected certain cases and illustrated them in Figs. 26—30.

Figs. 26 and 27 show two ESs normally organized but for the egg apparatus. In both cases this is composed of three cells; in Fig. 26, however, two cells have developed into egg cells and one only into a synergid.

Of the latter, a fragment is to be seen above the two egg cells, the rest lies in the next section. In Fig. 27 all the cells of the egg apparatus have developed into egg cells so that the number of those present in this ES (which is somewhat younger than the preceding one) is no less than three. A couple of additional ESs with supernumerary egg cells have been found.

Fig. 28 represents a more abnormally organized type. The ES-

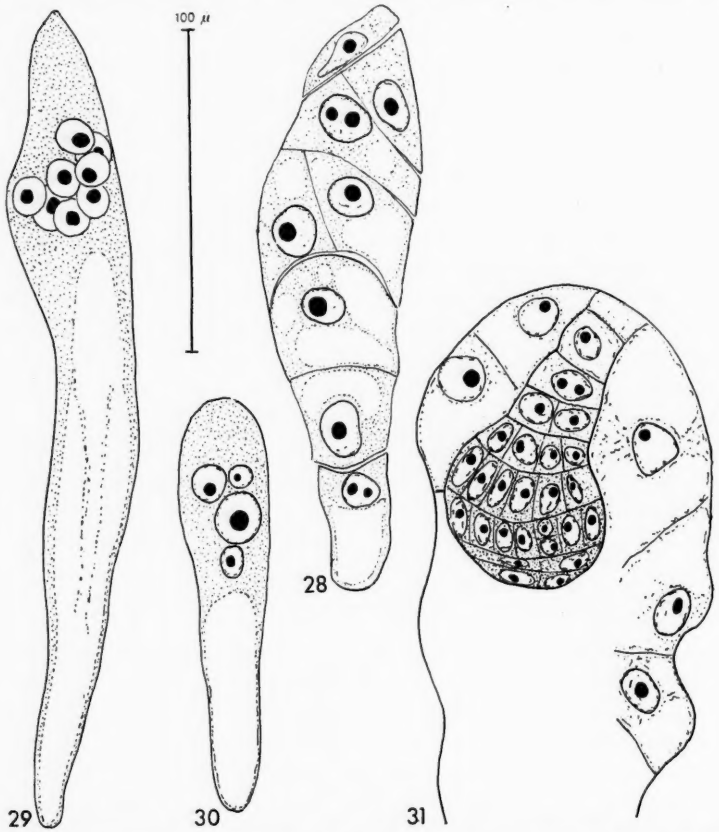


Figs. 25—27. *Antennaria carpatica.* — 25, normally organized ES. — 26, abnormal ES with two egg cells and one synergid a fragment of which appears in the upper part of the ES. — 27, abnormal ES with three egg cells.

cavity contains an eight-nucleate formation having one nucleus in every cell. Probably from the beginning it has been a question of an eight-nucleate ES in which cell walls between all nuclei have been formed, giving the whole an endosperm-like appearance. The case in Fig. 29 is quite the opposite of that in Fig. 28. Here eight nuclei are placed in the

upper part of the ES and there has been no wall formation at all, in spite of the fact that this ES lies in an ovule which on account of its size must be older than the former.

Finally, in Fig. 30 we have a young ES with four nuclei in its



Figs. 28—31. *Antennaria carpatica.* — 28—29, two abnormal eight-nucleate ESs, the one with walls between every nucleus, the other with no walls at all. — 30, four-nucleate ES with nuclei of different sizes probably originated from a tetrad with no wall formation. — 31, the single embryo which was observed by examining hundreds of mature ovules.

micropylar end. Apparantly there is a considerable difference in size between the nuclei. The origin of this ES is uncertain, due to the presence of the small nuclei. Most likely, however, it is a question of a

bi- or tetrasporous ES formed through an irregular meiosis. If wall formation during sporogenesis in the ovule of Fig. 3 or 4 had failed for some reason or other, I think that these could have given rise to a similar case.

IV. EMBRYO FORMATION.

In my previous paper on *A. carpatica*, I pointed out (1935 a, p. 221) that no embryos occurred in the old ovules but only degenerated or collapsed ESs. The re-examination involved in the present work, during which I examined hundreds of ovules, brought to light only one single embryo. All the other ovules were sterile. The embryo in question, shown in Fig. 31, appears entirely normal and is of a type common in the Composites. The endosperm, too, seems normal. Whether this embryo was formed sexually in a ± reduced ES or parthenogenetically in an unreduced one cannot be decided. At present it can only be stated that *A. carpatica* is practically sterile giving fertile achenes only on rare occasions. This corresponds to a certain extent with the rare occurrence of the plant in Scandinavia.

Finally I will deal with another peculiarity found in this species. In several ovaries, the ovule itself turned out to be abnormally developed. Sometimes it was divided so that two ovules occurred in the same ovary, sometimes it was abnormally orientated. By investigating an extensive material I have earlier on rare occasions observed similar abnormalities in other Composites, never, however, so frequent as in this *Antennaria* species. Practically in every head one or several such cases occurred. In certain ovaries (sometimes in all of the head) no ovules on the whole were developed in the ovary cavity; instead this was filled with an undifferentiated vegetative tissue having a peculiar undulated structure.

V. DISCUSSION.

The present investigation of the reproduction of *A. carpatica* has given unexpected results in at least three different respects: (1) meiosis, (2) gametophyte formation, (3) embryo formation.

Up till now regarded as a good species, this plant has by a closer examination revealed a meiosis which reminds one very much of that of a hybrid. It is impossible to decide which the ultimate parents could be, especially as *A. carpatica* differs considerably from the other species of the genus. However, there is also the possibility that it is a question of

an intraspecific hybrid to which the multivalent formation points. The lowest chromosome number occurring in the *Antennaria* species of the Old World is $2n = 28$ and is only represented in *A. dioica*. Hence, with its $2n = 40—42$, *A. carpatica* should most nearly be triploid. This applies only to the Scandinavian material, however, and very likely other chromosome numbers may occur in the rest of its distribution area. *A. carpatica* has partly a very northern distribution area in Europe, *viz.*, the most northern part of Scandinavia and Russia, and partly a more southern distribution in the mountain region of Central Europe from the Carpathians on the east to the Pyrenees on the west. It would not be very surprising if the representatives of these two widely separated distribution areas differed from each other in their chromosome number. In any case they seem to differ as to their fructification. As has been pointed out earlier, the investigated material of the Scandinavian *A. carpatica* has turned out to have practically empty achenes. However, fruit samples which Professor WALO KOCH in Zürich has been kind enough to send me from various localities in the Swiss Alps, show in several cases a very good fructification with apparently well-developed achenes. A well-developed achene is also illustrated in HEGI: »Illustrierte Flora von Mittel-Europa». This is in agreement with the fact that *A. carpatica* occurs much more abundantly in its alpine distribution area than in its North-European. As male and female individuals seem to be just as common in the Alps, this would seem to indicate a sexual reproduction while the relatively good achene formation points to a more balanced chromosome number. As mentioned above, the Scandinavian relatives are triploid with $2n = 40—42$ and therefore it is not impossible that the alpine plants like *A. dioica* are diploid with $2n = 28$. It would be very interesting to know the chromosome number and the reproduction conditions in the Central European *A. carpatica* and I am planning to make such an investigation.

Also unexpected was the remarkably high frequency of restitution nuclei in macro-sporogenesis. Since a corresponding nucleus formation failed to appear in micro-sporogenesis, I suggested that a special factor causing the formation of restitution nuclei must be present, this factor being active only in the ovules. This is very reminiscent of conditions in the agamosperms, several of which, *e. g.*, *Taraxacum*, *Erigeron*, *Chondrilla*, exhibit meiotical differences between EMCs and PMCs. Like these, *A. carpatica* forms its unreduced ESs through diplospory, although the egg cells lack the capacity of parthenogenesis.

Unreduced ESs in *A. carpatica* can also be formed through somatic

apospory. In the genus *Antennaria*, the European *A. alpina* and a great number of North-American species, reproduction is agamospermous. Agamospermous reproduction is also the rule in the South-American species, *A. magellanica* (BERGMAN, 1937). None of these, however, form their unreduced ESs through somatic apospory; instead, all ESs originate from the legitimate EMC which develops directly into the one-nucleate ES, the first division of which is a mitosis (generative apospory, according to FAGERLIND).

In two papers dealing with *Antennaria*, however, statements occur which can be interpreted as indicating somatic apospory. In both cases it is the question of sexual species. The oldest statement originates from HABERLANDT (1923) who, while making an embryological investigation of *A. dioica*, came across some heads in which chalazal cells developed into large vacuolisized cell-bladders containing several nuclei. The nuclei had large nucleoli and gave the impression of being ESs nuclei. Judging from the figures he published, there is scarcely any doubt that here is a case of somatic apospory of the same type as occurs in *Leontodon hispidus* (*cf.* BERGMAN, 1935 b, pp. 277—278). The second example is reported by STEBBINS (1932) who in a spontaneous hybrid between the sexual *A. neglecta* and *A. plantaginifolia* came across a single case of somatic apospory.

Evidently the tendency to somatic apospory is present in this genus. It is remarkable, however, that in those species where a real agamospermous reproduction occurs, this has taken the shape of generative apospory. However, as has earlier been pointed out by some workers (*cf.* FAGERLIND, 1944), in several cases it is very difficult to draw a line between these two types of apospory. Even in tenuinucellate plants with unicellular archesporium intermediate forms can appear (*cf.* BERGMAN, 1935).

Unexpected was also the proximately total seed sterility which proved to be characteristic of the Scandinavian *A. carpatica*. In hundreds of mature ovules examined only one single embryo was found. Hence it follows that *A. carpatica* gives real achenes only very rarely. Certainly its rare appearance in the Scandinavian mountains is to some extent due to this circumstance. The species is dioecious and often appears in small groups of exclusively male or exclusively female individuals, each group often growing at a considerable distance from the others. Probably each of these isolated groups has arisen from a single seed and developed vegetatively by branching. For such a group of individuals developed from one original seedling with the aid of a branching root or rhizome,

often in connection with rosettes, runners, *etc.*, HYLANDER has *in litt.* proposed the term rhizoclone, a term which the present author considers very appropriate and useful. These male and female rhizoclones of *A. carpatica* are of about the same frequency which would indicate that achene formation when it occurs has probably originated in a sexual way. However, sexual reproduction must be hindered, not only by the distances existing between the rhizoclones, but by other factors which probably make it even more difficult. As early as 1900, SKOTTSBERG studied the flower biology of, *i.a.*, *A. carpatica* in Swedish Lappland. Comparing it with *A. dioica* and *alpina*, he found certain peculiarities. He reports:

»*A. dioica* wird besonders von Schmetterlingen besucht; ihre rosa und weiss gefärbte Blütenköpfchen sind in ziemlich hohem Grade anlockend; Honig gibt es auch. *A. dioica* geht nicht besonders hoch auf die Berge hinauf. — Männliche Blüten sind bei *A. alpina* eine grosse Seltenheit; durch JUEL's Untersuchungen wissen wir, dass *A. alpina* parthenogenetisch ist. — Aber wie verhält es sich mit *A. carpatica*? Die bräunlich schwarzen Köpfchen rufen gar keine Aufmerksamkeit hervor; Honig habe ich in den Blüten nicht gesehen. Und in Lule Lappmark wenigstens wächst *A. carpatica* im oberen Teil der Weidenregion, oft viel höher, wo das Vorkommen von Insekten beinahe die reine Zufälligkeit ist. Wäre die Fortpflanzung bei *A. carpatica* von Insekten abhängig, würde die Art nicht lange bestehen können. Von Autogamie kann man natürlich hier nicht sprechen. Es ist ja nicht unmöglich, dass Anemophilie in diesem Falle vorkommen kann. Das auf den ♂-Köpfchen ausgebreitete Pollen kann, vom Winde geführt, in den Pappus der ♀-Köpfchen anhaften und so allmählich auf die Narben gelangen. — Leider habe ich nicht die Gelegenheit gehabt, die Fruchtbarkeit bei dieser Pflanze zu beobachten.» (SKOTTSBERG, 1900, p. 7.)

SKOTTSBERG's suspicion that there is something peculiar in the reproduction of *A. carpatica* has been well confirmed by the present investigation.

The proximately total seed sterility of the Scandinavian *A. carpatica* is very reminiscent of another Composite with agamospermous tendencies, namely, *Artemisia nitida*. According to CHIARUGI (1926), this species is a preglacial or interglacial relic in the Alps. Like *A. carpatica* it is triploid, and like *A. carpatica* it forms ± reduced ESs and unreduced ESs through diplospory or somatic apospory. Similar to *A. carpatica* it lacks the capacity of parthenogenesis and according to CHIARUGI it is totally sterile. It reproduces only vegetatively through rhizomes and consequently forms rhizoclones. Evidently, *Artemisia nitida* and *A. carpatica* are two parallel cases, and analogous to the conditions of *A. carpatica* it is not quite out of the question that *Artemisia nitida* can also develop achenes on rare occasions, thus enabling it to hold its position in its distribution area.

SUMMARY.

Antennaria carpatica, from three different localities of the Scandinavian mountains, has been cyto-embryologically investigated and the following results have been obtained:

(1) The meiosis is irregular with several univalents and multivalents.

(2) Micro-nuclei, triads, and polyads are formed both in macro- and micro-sporogenesis.

(3) In macro-sporogenesis, restitution nuclei are formed remarkably often. No such nuclei have been observed in micro-sporogenesis.

(4) A special factor must be present which causes formation of restitution nuclei. However, this factor is active only in the ovules.

(5) Three different types of embryo sacs are formed: (a) ± reduced, (b) unreduced through restitution nuclei (diplospory), (c) unreduced through somatic apospory.

(6) The unreduced embryo sacs lack the capacity of parthenogenesis.

(7) *Antennaria carpatica* is proximately totally seed sterile (only one single embryo has been observed) and is in several respects a parallel case to *Artemisia nitida*.

LITERATURE CITED.

1. BERGMAN, B. 1935 a. Zur Kenntnis der Zytologie der skandinavischen *Antennaria*-Arten. — Hereditas XX.
2. — 1935 b. Zytologische Studien über die Fortpflanzung bei den Gattungen *Leontodon* und *Picris*. — Svensk Bot. Tidskr., Bd. 29.
3. — 1937. Eine neue apomiktische *Antennaria*. — Svensk Bot. Tidskr., Bd. 31.
4. — 1944. A contribution to the knowledge of the embryo sac mother cell and its development in two apomicts. — Svensk Bot. Tidskr., Bd. 38.
5. — 1950. Meiosis in two different clones of the apomictic *Chondrilla juncea*. — Hereditas XXXVI.
6. CHIARUGI, A. 1926. Aposporia et apogamia in *Artemisia nitida* (BERTOL.). — Nuovo giorn. bot. Ital., N. S., 33.
7. FAGERLIND, F. 1937. Embryologische, zytologische und bestäubungsexperimentelle Studien in der Familie Rubiaceae nebst Bemerkungen über einige Polyploiditätsprobleme. — Acta Horti Bergiani, Bd. 11.
8. — 1940. Die Terminologie der Apomixis-Prozesse. — Hereditas XXVI.
9. — 1944. Die Samenbildung und die Zytologie bei agamospermischen und sexuellen Arten von *Elatostemma* und einigen nahestehenden Gattungen nebst Beleuchtung einiger damit zusammenhängender Probleme. — Kungl. Sv. Vet. Akad. Handl., S. 3, Bd. 21, N:o 4.

10. FAGERLIND, F. 1947 a. Macrogametophyte formation in two agamospermous *Erigeron* species. — Acta Horti Bergiani, Bd. 14.
11. — 1947 b. Makrosporogenesis und Embryosackbildung bei agamospermischen *Taraxacum* Biotypen. — Svensk Bot. Tidskr., Bd. 41.
12. GUSTAFSSON, Å. 1939. The interrelation of meiosis and mitosis. I. The mechanism of agamospermy. — Hereditas XXV.
13. HABERLANDT, G. 1923. Über die Ursache des Ausbleibens der Reduktionsteilung in den Samenanlagen einiger parthenogenetischer Angiospermen. — Sitzb. d. Preuss. Ak., Bd. XXV.
14. HEGI, G. 1906—1931. Illustrierte Flora von Mittel-Europa. — München.
15. ISHIKAWA, M. 1911. Zytologische Studien von Dahlien. — Bot. Mag., 25. Tokyo.
16. JUEL, H. 1900. Vergleichende Untersuchungen über typische und parthenogenetische Fortpflanzung bei der Gattung *Antennaria*. — K. Sv. Vet. Ak. Handl., Bd. 33, N:o 5.
17. MAHESHWARI, P. 1950. An introduction to the embryology of Angiosperms. — New York.
18. ROSENBERG, O. 1907. Cytological studies on the apogamy in *Hieracium*. — Bot. Tidsskr., 28.
19. — 1930. Apogamie und Parthenogenesis bei Pflanzen. — Handb. d. Vererbungswissenschaft, Bd. 2: 1. Berlin.
20. SKOTTSBERG, C. 1900. Einige blütenbiologische Beobachtungen im arktischen Teil von Schwedisch Lappland 1900. — Bih. t. K. Sv. Vet. Ak. Handl., Bd. 27, Afd. III, N:o 2.
21. STEBBINS, G. L. JR. 1932. Cytology of *Antennaria*. I. Normal species. — Bot. Gaz., Vol. XCIV, No. 1.
22. — 1941. Apomixis in the Angiosperms. — Bot. Rev., Vol. 7, No. 10.

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FORM AND BIOTYPE FORMATION IN CALAMAGROSTIS PURPUREA

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IN northern Europe and northwestern Siberia *Calamagrostis purpurea* (TRIN.) TRIN. is one of the most polymorphous species of the grass family. The species constitutes the eastern part of a big complex, the other components of which are named *C. Langsdorffii* (LINK) LINK and *C. canadensis* (MICHX.) BEAUV., and which are distributed from western Europe through northern Asia to the American east-coast. *C. purpurea* is an apomict with EMC's which develop diplosporously according to the *Antennaria* scheme. The species is an obligate apomict, needing no pollen for the development of the endosperm, and thus being non-pseudogamous. In 174 investigated clones from different parts of Sweden with chromosome numbers running from $2n = 56$ to $2n = 91$ (*cf.* NYGREN, 1946, p. 202) there was only one found with pollen. In all other cases meiosis in the anthers failed completely, or was severely disturbed, which caused big plasmodia only to be formed in the loculi. The clone with pollen was collected at Gällivare in northernmost Sweden and could only be separated from other *purpurea* clones in the pollen character. In 1943 the plant developed only two panicles, all florets of which had EMC's with meiosis instead of a diplosporous development characteristic of other *purpurea* clones. Next summer the plant yielded a large number of panicles, the earliest of which had meiosis in the EMC's as well as in the PMC's, while the EMC's in the panicles growing up later on developed diplosporously. In the summers of 1944—1946 a number of baggings and crosses were worked out on the Gällivare plant. No seeds were obtained after bagging the first developed panicles, while, on the other hand, seeds obtained from the later developed panicles gave rise to maternal offspring. Thirteen out of twenty-five crosses between the Gällivare clone and different amphimictic European species succeeded in 1944 (*cf.* NYGREN, 1946, p. 232), and ten out of eighteen in 1946 (*cf.* NYGREN, 1949, p. 292). In both years most hybrids formed had chromosome numbers about $2n = 70$, *i. e.* an unreduced female gamete from *purpurea* ($2n = 56$) had fused with a reduced gamete from the amphimictic parent

($2n = 28$). In 1944, as well as in 1946, some haploids with the number $2n = 28$ were obtained, which shows that some egg cells in this clone are able now and then to develop autonomously, in spite of the fact that they are reduced. In addition to this, three descendants with the chromosome numbers $2n = 43$, 45, and 48, have been obtained, which indicates that in these cases a reduced male gamete has fertilized a reduced female gamete. None of these last-mentioned plants, however, survived the second season.

In 1943 forty *C. purpurea* clones with the chromosome number of $2n = 56$ were selected and bagged. Twelve descendants of most plants were planted in blocks, while in six cases the number of seedlings planted ran up to between 16 and 57 (*cf.* NYGREN, 1948 a, p. 128). All 673 descendants had the same chromosome number as their original mother clones, or $2n = 56$. When they flowered in the summer of 1946 no morphological differences could be discovered on comparing them with the original clones, but it was revealed that 31 of the descendants belonging to eight clones had pollen, in spite of the fact that neither their parents nor the sister plants in the same block had any pollen. These 31 plants were dug up, planted in big pots, and placed far from other *Calamagrostis* cultures. All plants were bagged, and in most cases they were also crossed with different amphimictic species, just as was the case with the Gällivare plant.

Of the 31 plants in question, offspring have been raised from 59 baggings and 66 crosses. When the chromosome numbers of the descendants raised after bagging were counted, it appeared that different baggings had given diverse results in divergent panicles (*cf.* NYGREN, 1949, pp. 287—8). Thus sometimes all descendants from one and the same panicle of a certain clone plant have the same chromosome number as the mother clone, while those from another panicle on the same plant have higher numbers than the mother clone. The high numbers no doubt indicate that in these cases the plant's own pollen has functioned. The fact that the pollen may function in these *purpurea* plants should be noticed, because by contrast pollen of the Gällivare plant has not, hitherto, been functional. An embryological investigation, worked out in 1948, proved that some EMC's in the 31 »pollen plants» developed sexually, while others developed diplosporously according to the *Antennaria* scheme.

In the autumn of 1948 the *purpurea* seedlings from the 31 pollen plants were planted in the experimental field at the same time as five descendants from each of 41 new clones, raised from seeds obtained

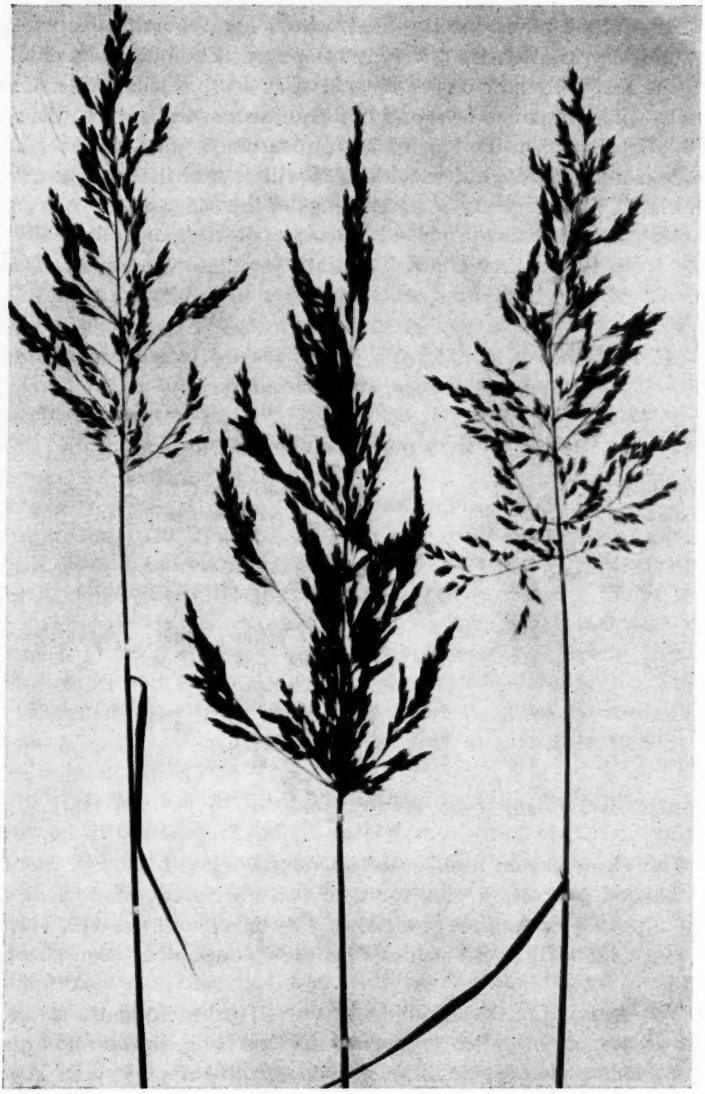


Fig. 1. Deviating offspring in the second generation of bagged *Calamagrostis purpurea*. The mother plant 99—1—4, originating from clone Robertfors 1—41 A, in the centre. Two offspring plants to the left and to the right.

after bagging of mother plants from different parts of Sweden. In the summer of 1950 all plants flowered, which made possible an examination of the variation in different offspring. The offspring obtained from the forty-one new clones did not vary — they were all maternal —, nor could any pollen be found in the anthers of these plants. In some of the offspring from the bagged 31 »pollen plants», on the other hand, there was a morphological variation, as will appear in Table 1, as well as in Fig. 1. The offspring of 59 baggings of the 31 »pollen plants» have been tested. Six clones produced progeny, which morphologically deviated from the mother clone. The data for these are summarized in Table 1.

TABLE 1. *Variation in offspring in the second bagged generation of Calamagrostis purpurea.*

Mother clone	Panicle No.	Total of plants	Chromosome number of second generation plants	Morphological deviations or non-deviations from the original clone
Sylarna 1—2	402	5	76, 80 (3 plants not det.)	Deviating
Östersund 3—3	459	5	56, 56 (3 plants not det.)	»
Dorotea 1—3	419	2	56 (1 plant not det.)	»
1—4	417	4	56, 56, 56 (1 plant not det.)	»
2—3	412	5	56, 58, 70 (2 plants not det.)	»
2—3	441	5	56, 56 (3 plants not det.)	Not deviating
2—3	457	5	68, 69, 70, 70 (1 plant not det.)	»
3—2	456	4	63, 64, 68, 68	Deviating
Gittafjäll 2—3	454	5		»
Jormlien 2—4	429	5	70, 71, 71 (2 plants not det.)	»
Robertsfors 1—4	426	5		»

The chromosome number of all mother clones, as well as of all first bagged generation plants, was $2n = 56$. No morphological variation appeared in the first generation. The three numbers 412, 441, and 457 mean three different panicles on the same mother plant, Dorotea 2—3.

The reason for the variation in the offspring from the 31 clones is the occurrence of pollen in some of the first bagged generation plants, because this pollen no doubt is able to fertilize the egg cells in some of the ES's of the same plant and thus cause the development of descendants with a variation in the chromosome number. It is really a unique situation that up to date only 31 plants with pollen have been

found in 878 descendants from 81 bagged clones of the species, but this may depend upon the fact that the environment plays a great rôle in the formation of pollen in *Calamagrostis purpurea*, for the species is very labile in this respect. It is also true that the amount of pollen in the loculi is greater in the first year the plant flowers, while it decreases during the following seasons at the same time as there is an increase in the formation of plasmodia. This has been proved for the Gällivare clone, which has been in cultivation since 1941, as well as for most of the pollen plants revealed in the offspring from the clones bagged in 1944.

In 1944 a number of crossings were made between the Gällivare *purpurea* plant and different amphimictic species. The main chromosome number in the hybrids obtained was $2n = 70$ (NYGREN, 1946, p. 232), and all F_1 plants showed very slight morphological deviations from the mother clone (NYGREN, 1948 a, pp. 122—3). The same conditions have now been shown to be true of hybrids formed after crosses made on the Gällivare plant in 1946. These hybrids have been morphologically checked in 1950, while their chromosome numbers were determined in 1949 (NYGREN, 1949, p. 292). In the summer of 1950 the hybrids formed between some of the 31 »pollen plants» of *purpurea* and different amphimictic species were also checked. Seven of the 31 clones had given hybrids (*cf.* NYGREN, 1949, p. 290) in 15 different combinations. Four of these offspring deviated morphologically from the mother clone, and in some cases also the sister plants in one and the same cross differed from each other. Great variation occurred in the F_1 especially in one cross with *C. arundinacea* as father, and in two others with *C. canescens*.

In 1950 the F_2's of the 1944 crosses between the Gällivare *purpurea* and different amphimictic species were checked. Offspring were obtained from fifteen different combinations with five amphimictic species. The F_2 plants deviated in practically every case from the F_1 plants, in spite of the fairly small number of plants available (56 in all). One cross with *C. canescens* as father gave nine F_2 plants, which all deviated from each other (*cf.* Fig. 2). Some of them showed great morphological resemblance to the amphimictic species *C. epigeios*. The offspring of another cross with *C. canescens* varied a great deal, as appears in Fig. 3. One of four surviving F_2 plants from a cross with *C. arundinacea* showed great morphological resemblance to the father (Fig. 4), but deviated in having longer callus hairs and in the lack of hairs, where the straw and the leaves meet each other. The chromo-

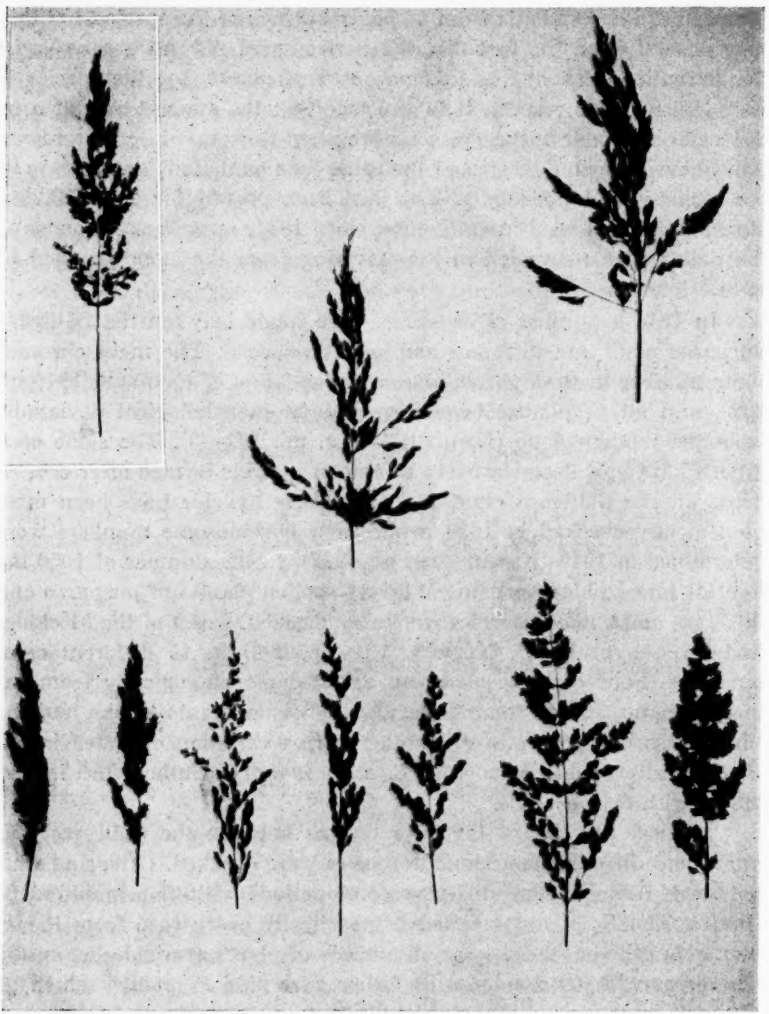


Fig. 2. The morphological pattern of a *Calamagrostis purpurea* × *canescens* cross. The *canescens* father ($2n = 28$) to the upper left, the *purpurea* mother to the upper right, F_1 hybrid ($2n = 70$) in the middle centre. All F_2 plants in the lowest row have chromosome numbers deviating between $2n = 34$ and $2n = 38$.

some number of the F_1 plants was $2n = 70$ in most cases, while the numbers of the F_2 plants have only been determined in two offspring.

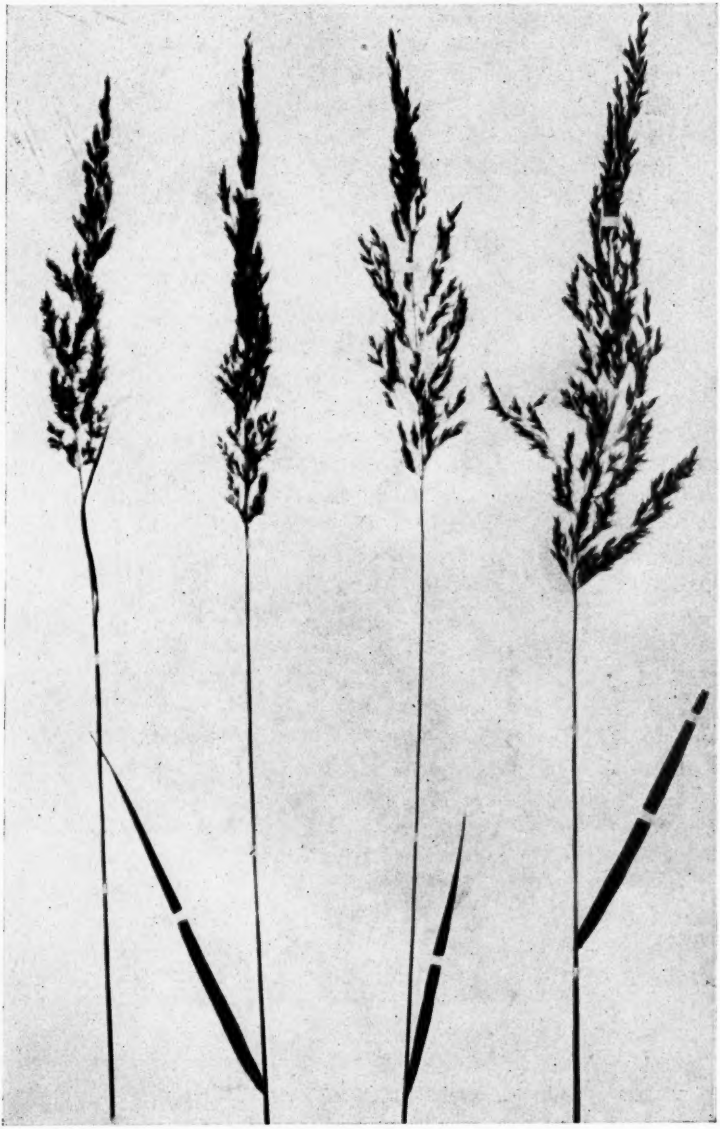


Fig. 3. Four different F_2 descendants from a cross between *Calamagrostis purpurea* and *canescens*.

Fig. 4. *Arundinacea*-like F_2 plant to the right compared with a sister plant of maternal type, both obtained in the second generation of a cross between *Calamagrostis purpurea* ($2n = 56$) and *arundinacea* ($2n = 28$).

The nine survival F_2 plants in the variable offspring of the first cross mentioned between *purpurea* and *canescens* have the chromosome numbers $2n = 34$, 35, 35, 35, 36, 36, 36, 37, and 38. The F_2 descendant that showed resemblance to *arundinacea* has $2n = 34$, while its sister plant, which looks like the F_1 hybrid, has $2n = 70$. The whole series with low chromosome numbers in the first *canescens* cross is remarkable, but probably depends upon a combination of sexuality and haploid parthenogenesis. The Gällivare clone of *purpurea* is now and then known to give rise to haploids, which apparently develop by haploid parthenogenesis. The F_1 plant in the *canescens* cross has the capacity to develop diplosporous as well as sexual ES's, just as have all other F_1 plants investigated in crosses between *purpurea* and amphimictic species. For some reason or other, only sexual ES's have developed seeds in this case, while in the *arundinacea* case sexual as well as diplosporous sacs have functioned.

The F_1 plants of the crosses between *C. purpurea* and different amphimictic species had been embryologically tested in 1948 (*l. c.*, p. 123). The test showed that in these plants sexual as well as diplosporous ES's occurred just as in *C. purpurea* itself, though the number of sacs, developing according to the *Antennaria* scheme, were in majority. A study of the F_2 plants of the variable cross with *C. canescens*, as well as of the single deviating F_2 plant in the *arundinacea* cross, made it clear that even these plants are able to produce sexual as well as diplosporous ES's. The facts have been summarized in Table 2.

In all these F_2 plants there was a development of plasmodia in the

TABLE 2. *Number of sexual and diplosporous embryo sacs found in F_2 of crosses between C. purpurea and amphimictic species.*

Combination	Plant number	Chromosome number $2n$	Number of sexual ES's found	Number of diplosporous ES's found
C. p. × *C. c.*	221—3—1	35	6	5
	2	36	3	9
	3	35	3	2
	4	38	4	5
	5	35	4	3
	6	36	3	4
	7	36	8	1
	8	34	—	—
	9	37	2	5
C. p. × *C. a.*	220: 2—1	34	2	3

loculi. Plant 221: 3—1 was the only one in which morphologically good pollen was found during the summer of 1950.

Embryo sacs with two or four nuclei, found in nucelli without any signs of degenerated tetrad cells, have been classified as diplosporous, while nucelli with normal triads or tetrads have been referred to the sexual group. In the plant 221: 3—3 chromosome counts have been made in nuclei of sexual as well as of diplosporous sacs. One reduced nucleus held 18 chromosomes, while an unreduced one had $\pm$ 35 bodies. The formation of a restitution nucleus has been observed in a first anaphase of the EMC of the plant 221: 3—7. In Nos. 221: 3—4 and 9, respectively, the autonomous development of egg cells has been noted.

It is clear from Table 2 that all tested F_2 plants — except 221: 3—8 in which no sure determinations could be made — have sexual as well as displosporous ES's and thus do not deviate from the F_1 plants in this respect. Unfortunately, the ratio between the number of different sacs cannot be evaluated from the figures in Table 2. In these diplosporous plants it is, however, possible only to classify either early stages or ES's, in which the chromosome number of the nuclei can be counted. Thus the number of sacs classified is very small in relation to the number of sacs studied. Although it has not been possible to calculate the ratio between diplosporous and sexual embryo sacs, it must be considered important to know that F_1 plants, which give rise to segregating offspring in F_2, are able to produce sexual as well as diplosporous ES's, and that this capacity has been transmitted to the F_2 plants tested.

CONCLUSIONS.

In many respects the genetical and morphological patterns in *Calamagrostis purpurea* are similar to those in the genera *Rubus* and *Poa*, and presumably to those existing in *Archieracium* and *Hieracium pilosella*. In *Calamagrostis purpurea* the rare facultatively apomictic plants produce the great variation commonly observed in the species either by self-fertilization or by hybridization. The diplosporous mechanism plays an important rôle in these types by its retardation of the division in the EMC, which makes a fertilization easier than in apomictic species which have an early development of the EMC. It seems therefore possible that also more or less obligate apomictic strains of the species would be able to form hybrids though until now no hybrids have been obtained by crossing such *purpurea* clones with amphimictic

species in the genus. The experiments with the forty *purpurea* clones, mentioned above, show, however, that the limit between obligate and facultative clones is sometimes very difficult to draw. This has been proved by the experiments with the Gällivare clone compared with those of the thirty-one pollen plants. Different facultative apomicts of *purpurea* also behave differently according to their capacity to form new biotypes. Thus in the case of Gällivare *purpurea*, after bagging, no aberrants were formed, while several of the thirty-one pollen plants yielded a high amount of aberrants. At the same time it has been shown that hybrids between the Gällivare *purpurea* and amphimictic species produced F_1 plants which were morphologically similar to the *purpurea* clone, while several of the thirty-one pollen plants gave rise to F_1 hybrids deviating from the *purpurea* mother. This deviation or non-deviation did not depend upon the chromosome number of the hybrids, for, in most cases checked, the hybrids had $2n = 70$.

Summarizing the data obtained, the following facts concerning the biotype formation in *Calamagrostis purpurea* have been proved:

(1) Facultative as well as obligate apomicts occur in nature. The obligate clones do not form pollen. Some clones lacking pollen, and because of that classified as obligate apomicts, have, after bagging, given rise to descendants which have pollen. When these descendants were bagged, they gave in some cases segregating offspring, while, in other cases they gave non-segregating offspring. Different bagged panicles on the same plant gave rise to diverse results in this respect. Thus one panicle gave deviating offspring, while another gave maternal. The deviating plants sometimes had chromosome numbers which were different from the mother plant, while, in other cases, they had the same chromosome number as the bagged plant. The aberrants thus originated partly from self-fertilization, partly from autosegregation (GUSTAFSSON, 1943).

(2) Facultative apomictic *purpurea* strains are able to produce hybrids with amphimictic species. No hybrids have been obtained in the crossing experiments with obligate apomictic clones. The hybrids formed may have the same appearance as the *purpurea* mother or may deviate. Different plants behave differently in this respect. Most hybrids have chromosome numbers between $2n = 70$ and $2n = 84$. In some very rare cases hybrids have been obtained with chromosome numbers lower than the *purpurea* mother.

(3) The F_1 plants gave rise to segregating offspring in F_2, and could, therefore, be interpreted as sexual. Embryological investigations

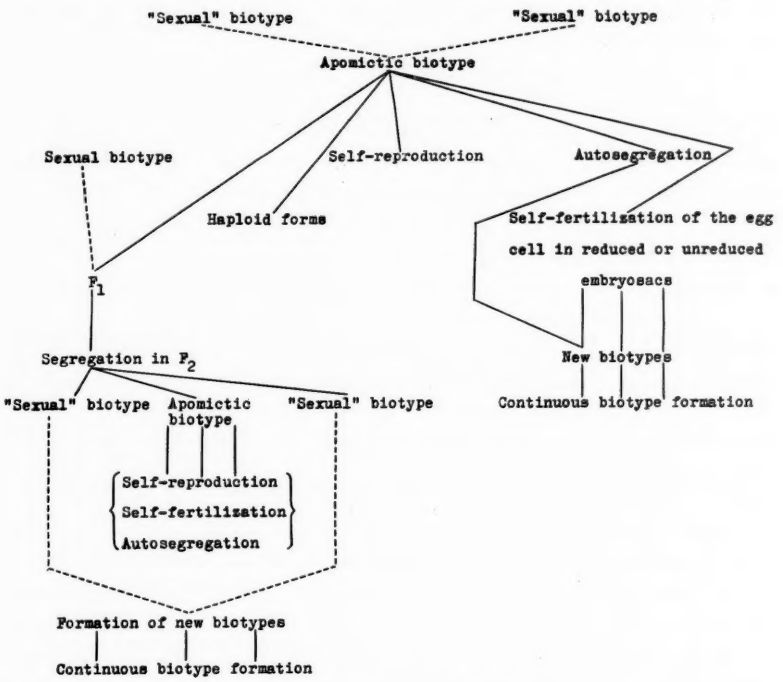
of the F_1 plants have proved, however, that diplosporous EMC's as well as meiotic EMC's appear in the nucelli of these plants, and, therefore, they should be classified as facultative apomicts and not as sexuals. The capacity to form two kinds of EMC's occurs in all F_2 plants embryologically tested except one, and therefore these plants, also, are to be interpreted as facultative apomicts.

(4) The *purpurea* clone from Gällivare forms occasional haploids, which proves that even egg cells in a reduced ES have the capacity to develop autonomously. This capacity has been transmitted to one of the F_1 plants.

(5) *Calamagrostis purpurea* has been synthesized in species crosses between *Calamagrostis epigeios* and *canescens* (NYGREN, 1946, 1948 a). This synthesis can now be explained in the following way. In nature *C. purpurea* may cross with different amphimictic species in its own distribution area. By this process *purpurea* genes — and at the same time genes for apomixis — are widely spread in the populations of different amphimicts. Thus many amphimictic species may be heterozygous for these genes of apomixis, and therefore *purpurea* will now and then be recombined in nature, when the fitting gene combinations are brought together. The *epigeios* plant, used by the author in the crosses, originated from southern Lapland in northern Sweden. In this area *purpurea* is the predominant species in the genus. The *canescens* plants used were also from parts of Sweden where *purpurea* is fairly common.

(6) The synthesis of *purpurea* as well as the results obtained in the crossing experiments suggest the possibility that genes for apomixis, originating from *purpurea*, occur in the entire *Calamagrostis* population within the distribution area of *purpurea*. Apomixis may therefore also be recombined in species complexes other than *purpurea*. This has also been proved in a cross between *Calamagrostis epigeios* ($2n = 56$) and *arundinacea* ($2n = 28$). In the F_1 plant itself, as well as in seven F_2 plants, diplosporous EMC's occurred together with normal sexual ones (NYGREN, 1946, p. 241; 1948 b, p. 406). It might therefore be possible to stabilize apomixis in this way in a complex of the genus where it has not previously been noted in nature.

(7) The combination of sexuality and apomixis in *Calamagrostis* makes possible a formation of a higher number of distinct forms in nature than does the usual sexual process. The facultative apomictic strains are able to perpetuate themselves by diplospory at the same time as they give rise to new biotypes by hybridizing with other facultative strains or with other species. *Calamagrostis purpurea* may be able to



Scheme 1. Biotype formation in *Calamagrostis purpurea*.

hybridize with sexual species adapted to climates outside its range. When the distribution areas of the different species overlap new forms will arise which are fitted to environments different from those to which *purpurea* is adapted. In this way the species complex may be able to spread into new areas. In the case of *Calamagrostis purpurea* it is not possible to keep the sexual and the apomictic formation of biotypes apart as has been done by several authors in other material. The two processes sometimes occur separately, but they often intermix, and in such cases great variation will be the final result.

(8) The same situation which occurs in *Calamagrostis* has also been demonstrated for the genus *Poa* (*cf.* CLAUSEN, KECK, HIESEY, and GRUN, 1949; ÄKERBERG, 1951; NYGREN, 1950).

(9) Excluding consideration of somatically produced mutations the biotype pattern in *Calamagrostis purpurea* follows Scheme 1.

Financial support has been received from Statens naturvetenskapliga forskningsråd.

LITERATURE CITED.

1. CLAUSEN, J., KECK, D. D., HIESEY, W. M., and GRUN, P. 1949. Experimental taxonomy. — Annual report of the Director of the Division of Plant Biology. Carnegie Institution of Washington, Year Book, No. 48: 97—103.
2. GUSTAFSSON, Å. 1943. The genesis of the European blackberry flora. — Lunds Universitets Årsbok. N. F. Bd. 39, No. 6: 1—200.
3. NYGREN, A. 1946. The genesis of some Scandinavian species of *Calamagrostis*. — Hereditas XXXII: 131—262.
4. — 1948 a. Further studies in spontaneous and synthetic *Calamagrostis purpurea*. — Hereditas XXXIV: 113—134.
5. — 1948 b. Some interspecific crosses in *Calamagrostis* and their evolutionary consequences. — Hereditas XXXIV: 387—413.
6. — 1949. Apomictic and sexual reproduction in *Calamagrostis purpurea*. — Hereditas XXXV: 285—300.
7. — 1950. Cytological and embryological studies in Arctic *Poae*. — Symbolae Botanicae Upsalienses X: 4: 1—64 + 15 pl.
8. ÅKERBERG, E. 1951. Studies of the F_2—F_4 progeny from the hybrid between *Poa pratensis* L. (coll.) and *Poa alpina* L. — Proceedings of the Seventh International Botanical Congress in Stockholm, 1950. (In the press.)

X-RAY INDUCED LETHAL MUTATIONS IN SEVERAL STRAINS OF DROSOPHILA MELANOGASTER

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INTRODUCTION.

IT is always found that a certain number of fertilized eggs in the stocks of *Drosophila melanogaster* do not hatch. The causes for the death of these zygotes have not as yet been fully explained. However, some of the zygotes are probably killed by dominant lethal mutations, either in the egg or in the fertilizing sperm. A dominant lethal mutation can be defined as such an alteration of the chromosomal material in the gamete that the zygote formed does not develop to sexual maturity.

MULLER (1927) showed that dominant lethals can be induced by radiation. Several investigators confirmed his findings and have made more detailed analyses. The literature on the subject is reviewed by CATCHESIDE and LEA (1945), who also point out that two kinds of discrepancies between different sets of data published are found. First there are investigators who find that the rate of induction of dominant lethals is constant per unit X-rays for all doses as contrasted to investigators who find that the induction of dominant lethals increases per unit X-rays with increasingly higher doses. The other discrepancy is found when a comparison is made of the rates of dominant lethals induced by the same dose of X-rays as observed by different authors.

The present author (1949) pointed out that some discrepancies of the latter class may be due to a variation in the age of the irradiated sperms. The older sperms show a higher percentage of dominant lethals than the younger ones when they are treated with the same dose of X-rays. This has been confirmed by BONNIER and LÜNING (1950), who also showed that the time from irradiation to fertilization had an effect. The longer the time interval, the lower was the proportion hatched. Several authors have suggested that different strains of *Drosophila melanogaster* may be expected to yield different amounts of dominant lethals when treated with equal doses of X-rays. In the present paper the author has taken this problem up for investigation.

MATERIALS AND METHODS.

Strains of *Drosophila melanogaster* were obtained from the Division of Genetics, and from Dr. C. STERN and Dr. L. K. PITERNICK (Department of Zoology) at the University of California, from the Carnegie Institution of Washington, Cold Spring Harbor, New York, and from Institute of Genetics, University of Oslo, Norway.

TABLE 1. *Wild type strains of Drosophila melanogaster. The table lists the numbers used by the author and the corresponding name of the strains employed. It also gives the sources these strains are obtained from.*

No.	Name	From
1A	Canton S	Division of Genetics, Univ. of California
2A	Oregon R-C	»
2B	Oregon	»
4A	Oak Glen, Riverside	»
5A	Corona	»
6A	Indio	»
7A	Sacramento	»
8A	Fresno No. 1	»
9A	Fresno No. 2	»
10A	Iso-Iowa	Dr. C. STERN, Dept. of Zool., U. of Calif.
11A	Iso-Crimea	»
12A	Iso-Seta-Japanese	»
13A	Iso-Amherst	»
14A	Iso-Argentine	»
15A	Iso-Princeton	»
16A	Iso-California	»
17A	Iso-Smarkland	»
18A	Iso-Kyoto	»
19A	Iso-Oregon	»
20A	Iso-Tuscaloosa	»
21A	Kenya	»
22A	$3 + ^3$	»
23A	Florida 10 iso	Dr. PITERNICK, Dept. of Zool., U. of Calif.
24A	Florida 26—24	»
25A	Jund, Berkeley	Mr. ERNEST JUND, Div. of Genet., U. of Calif.
26A	Formosa, Japan	Carn. Inst., Cold Spring Harbor
27A	Lausanne Special	»
28A	Salta, Argentine	»
29A	Swedish-b-6 (Swedish-b cleaned of inversions)	»
30A	Urbana Special	»
31A	Woodbury, N. Y.	»
32A	Bryn, Norway	Inst. of Genetics, Oslo University, Norway

Mr. ERNEST JUND, Division of Genetics, University of California, collected in Oakland, California, one female and two males which gave rise to strain No. 25A. This strain was received by the author four weeks after the first flies were trapped.

Table 1 lists the name and the number of the strains used and also the sources from which they were obtained.

It may be mentioned here that of the 32 strains listed in Table 1, 19 strains trace back to entirely different collections from the wild. The relationship among the remainder are as follows: 2A and 2B originated from the same Oregon R-C stock. However, strain No. 2A has been kept going by mass transfer, while strain No. 2B has been maintained by brother-sister matings with occasional half brother-sister matings since May 13th, 1941. The strains Nos. 10A to 22A which were originally the same Canton stock were made isogenic at the same time (two years ago) but with different fourth chromosomes. The names of these strains indicate the region from which the fourth chromosomes have come. These strains have been maintained by mass transfer from the time they were made isogenic until they were received by the author. Before these experiments started, strains Nos. 10A, 11A and 12A were inbred by brother-sister matings for 14, 16 and 17 generations, respectively. The strains Nos. 4A, 5A, 6A, 8A and 9A were inbred 36, 34, 35, 26 and 30 generations, respectively. All other strains not mentioned here were maintained by regular mass transfer.

Strain No. 6A was found to contain a wing mutant which affected all males and most females. It varied in expression from the loss of a few hairs on the wing margin to deep notches and did not appear to be an allele of vestigial. This mutant has not been fully investigated yet. In No. 26A several flies were found with vestigial wings and when virgin females expressing this character were crossed to *vg se* males they gave only vestigial offspring.

The females used as egg layers in all crosses were obtained by mating females of a strain homozygous for the genes *cn*, *bw* and e^{11} with wild type males of the 1A strain. These hybrid virgin females have been found to possess hybrid vigour and capacity to produce eggs with high hatchability when crossed to unirradiated males.

The cultures were raised in half-pint bottles containing the usual cornmeal-molasses medium, supplemented with one per cent brewers yeast. All cultures were raised at a temperature of $25° \pm 1°$ C.

Since the purpose of the study was to investigate the difference in sensitivity to X-rays of different strains, it was very important to keep

constant as many variables as possible. Therefore, the following procedure was adopted. Around 200 pairs of flies of each strain studied in a given experiment were put into a culture bottle, allowed to deposit eggs for 12 hours, and then discarded. Ten days later all flies which had emerged by that time were discarded, but all those emerging the next day were collected. Of these flies, the females were discarded, while the males were stored in regular culture bottles. These males had taken around ten days for developing. When the males were four days old they were subjected to X-ray treatment. Subsequent to the irradiation, the males were mated to the virgin hybrid females. The technique of egg collecting and egg counting was the same as previously described by the author (1949). The eggs collected were those deposited during 12-hour periods and usually for six consecutive days.

TYPES OF RADIATION AND MEASUREMENT.

A General Electric Maximar 220 kilovolt X-ray apparatus, made available through the courtesy of the WILLIAM H. CROCKER Radiation Laboratory, was used for all irradiations. The irradiations were performed jointly by Dr. EVERETT R. DEMPSTER and the author.

Many small doses were used so that each dose could be measured by a 250 r-unit thimble chamber of a Victoreen dosimeter. The tube was operated at 200 kilovolts, 10 milliamperes, with a 1,2 mm. aluminum and a 0,25 mm. copper filter. The flies, kept in small gelatin capsules (5×11 mm.) which had been pierced at each end, were placed at a distance of 33 cm. from the target, and given a total dosage of 2300 r-units of X-ray. This was accomplished by administering nine doses of approximately $246\,{}^1/_2$ r-units and one dose of 82 r-units. The number of males treated at one time with X-rays had to be limited, due to the time required to count the eggs fertilized by sperms from these males. It was, therefore, necessary to divide all the males into eleven groups for irradiation at different times. However, no dosage corrections seem to be needed, since all the dosage measurements lie within 2300 ± 3 r-units.

THE EXPERIMENTS, THEIR DATA AND STATISTICAL TREATMENT.

For purposes of analysis, the data are divided into four groups which will be referred to henceforth as experiments No. I to No. IV. In the first experiment 17 unrelated strains and in the second experiment

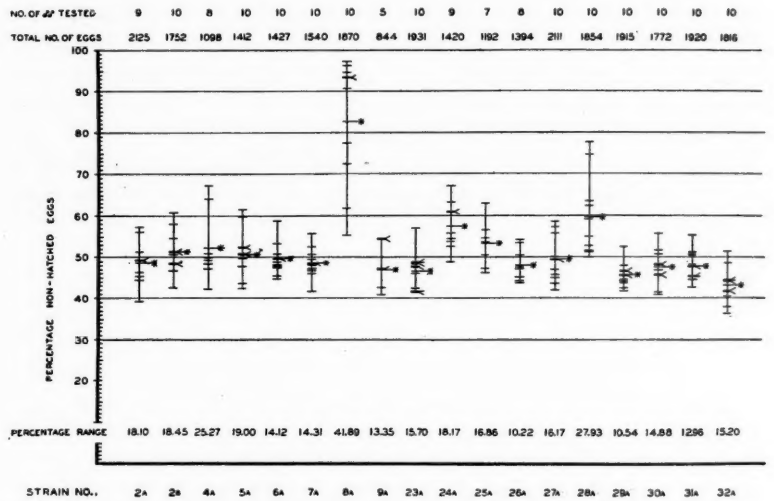

Fig. 1. Percentage non-hatched eggs observed in experiment I. The eggs were fertilized by sperms from different strains which were treated with 2300 r-units of X-rays. Horizontal lines indicate the observations for the individual males and the vertical lines connect observations for males belonging to the same strain. * indicates the mean for each strain.

13 related strains were tested with regard to their sensitivity to the induction of dominant lethal mutations by equal doses of X-ray. In the third experiment, nine strains selected from the two previous experiments were tested with regard to the hatchability of these strains when only untreated males were used. The fourth experiment had two purposes — one was to test the homogeneity of treatments on different days and the other was to observe the hatchability of eggs deposited on the first to the third and on the fourth to the sixth days after irradiation.

Statistical analyses of the data were carried out to test the significance of the difference in frequency of non-hatched eggs observed in the two parts of the total egg-laying period of six days, and also in order to obtain a measure of the within and between strain and treatment variations.

Experiment I. — Males from 17 unrelated strains were given an X-ray treatment of 2300 r-units. The males had taken ten days for their development, were four days old, and had been stored without females. Subsequent to the irradiation they were, as already noted, mated to virgin females heterozygous for *cn*, *bw* and e^{11}. The males were kept

with the females during the whole experiment. The total number of eggs deposited by each single pair culture was recorded and the percentage of non-hatched eggs calculated. In this experiment 29393 eggs were counted. Fig. 1 presents in a condensed form the data observed in this experiment.

Experiment II. — In this experiment, 13 variants (strain 10A to 22A) of originally the same stock were tested. They were all made iso-

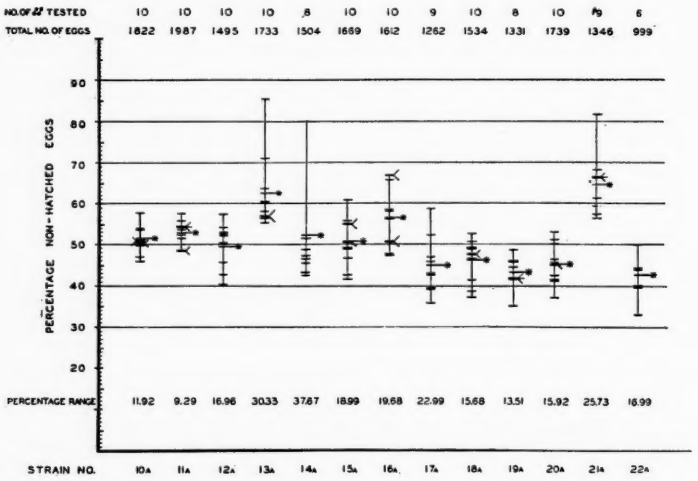

Fig. 2. Percentage non-hatched eggs observed in experiment II. The eggs were fertilized by sperms from different strains which were treated with 2300 r-units of X-rays. Horizontal lines indicate the observations for the individual males and the vertical lines connect observations for males belonging to the same strain. * indicates the mean for each strain.

genic at the same time, the only difference between them being in the fourth chromosome (see Table 1). It might not be an unreasonable assumption that, at the time when these variants were made isogenic, the variation that would have been observed between different males of the original stock would be chiefly due to chance alone. Hence, any variation between these strains beyond that due to chance might be attributed to differences between their fourth chromosomes together with possible genetic differentiation stemming from mutation or from partial failure of the procedure used to produce isogenicity. Males from these strains, which had been maintained by mass transfer for two

years, were given an X-ray treatment of 2300 r-units and then mated to hybrid virgin females, as was done in the previous experiment. A total of 20033 eggs was counted. A summary of the observed data is presented in Fig. 2.

Experiment III. — A control series of strains was examined without X-ray treatment. The nine strains compared were chosen on the basis of the results in experiments I and II, five being the ones with the highest and four with the lowest percentage of non-hatched eggs in

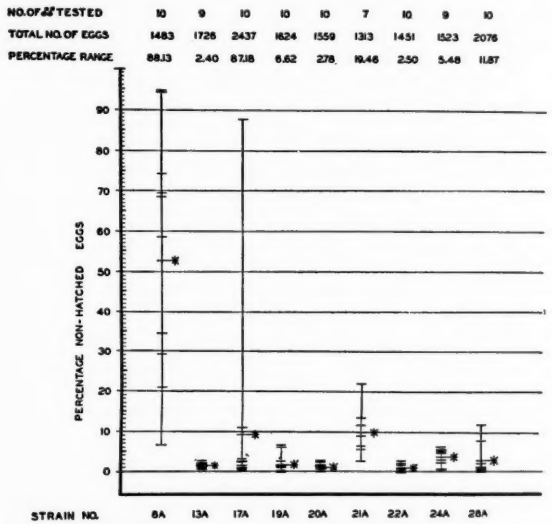

Fig. 3. Percentage non-hatched eggs observed in experiment III. The eggs were fertilized by untreated sperms from different strains. Horizontal lines indicate the observations for the individual males and the vertical lines connect observations for males belonging to the same strain. * indicates the mean for each strain.

these experiments. The procedure followed was exactly the same as in previous experiments, except that the males were untreated. A total of 15192 eggs was counted in the control series. The data are presented in a condensed form in Fig. 3.

Experiment IV. — A critical review of the data of experiments I and II revealed that several irradiated on a given day sometimes tended to show a higher or a lower percentage of dominant lethals than the average for the whole series. Thus, strains Nos. 10A and 11A were irradiated on the same day and gave respectively 51,37 and 52,84 as the

mean percentage of non-hatched eggs. Strains Nos. 13A, 16A and 21A were irradiated on another day and gave as the mean percentage of non-hatched eggs 62,49, 56,38 and 64,78, respectively. On a third day, strains Nos. 17A, 19A and 22A were irradiated and they gave respectively 45,09, 43,20 and 42,54 as the mean percentage of non-hatched eggs.

In order to test the variation from one day's X-ray treatment to another, these eight strains were used. Five males from each strain were irradiated simultaneously and then mated to virgin hybrid females as described in the previous experiments. The only difference here was that in this case the eggs counted were all those eggs which were laid during the first three days, both day and night. One week later, another five males from each of the same strains were irradiated and then mated to virgin hybrid females. From these females all the eggs deposited during the day and the night for the first three days were recorded. These females were allowed to deposit eggs for another three days and a separate record was made of these eggs. The data from the first set of five males are recorded as: Repeat A, and the data for the next set of five males are recorded as: Repeat B, when only eggs laid during the first three days are considered and as: Repeat C when the eggs laid during the three following days are considered.

In this experiment, 25418 eggs were counted. The mean percentage of non-hatched eggs for each strain is presented in Fig. 4.

It may be noted, before going into the details of the statistical analyses, that strain No. 8A will be excluded from all statistical treatments. The reason for this procedure is that eggs fertilized by untreated males of this strain show very poor hatchability (mean value 47,40 per cent) as well as great variability. This variability, as may be readily seen from Fig. 3, is so extreme as to render entirely uncertain the effect attributable to radiation in the treated series. By contrast, the eggs fertilized by most of the untreated males among the other strains showed hatchabilities greater than 97 per cent, the mean value being 96 per cent (see Fig. 3). This great variability among the controls of strain 8A is, as would be expected, reflected in a corresponding variability among the treated flies, the range being 41,89 per cent as compared to a mean range of 16,54 per cent for the other strains listed in Fig. 1. No other selection of the data, than the one mentioned above, was made except that completely sterile cultures and cultures with abnormal eggs (as noted in the discussion) were excluded.

Chi square tests were made to determine whether the variation between the first and the second half of the egg laying period, the within

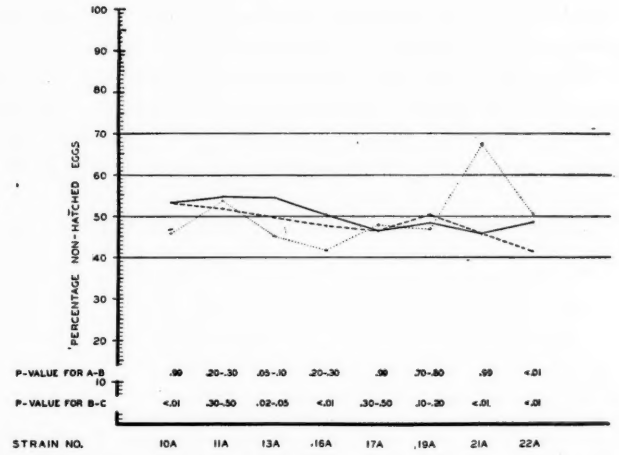

Fig. 4. Mean percentage non-hatched eggs observed in repeat A, repeat B and repeat C. ——— connects mean values observed for the different strains in repeat A. - - - - - - connects mean values observed for the different strains in repeat B. ..... connects mean values observed for the different strains in repeat C. The P-values entered on the figure are obtained by chi square analyses of the difference for the individual strains between repeat A and B and of the difference observed between repeat B and C.

strain, and the between strain variations might be due to chance. The following interpretation of the P values was adopted: a P smaller than 0,01 indicates variation greater than would be expected due to chance alone, a P value larger than 0,05 indicates that the variation is no greater than would be expected due to chance, and P values in the range from 0,01 to 0,05 are considered to belong to the doubtful range.

THE FREQUENCY OF NON-HATCHED EGGS RELATED TO TIME OF EGG LAYING.

Since, in experiments I and II, only the eggs deposited during the day on six consecutive days were counted, while in repeat A and B (experiment IV) all eggs laid during three days and nights were counted, it was considered desirable to extend repeat B for another three days and nights and record the data observed for this last period as repeat C.

A statistical analysis of the difference between the data observed in repeat B and in repeat C was carried out, and the P-values obtained listed in Fig. 4. It can be seen that the difference in hatchability between eggs laid on the first three days and eggs laid on the three following

days is significant for four of the strains, three of the strains do not vary more than expected due to chance, while one strain falls into the doubtful class. Since half of the strains investigated here show a significant difference in hatchability between the eggs laid during the first three days and those laid during the following three days, it was found necessary to apply a detailed analysis of the data recorded in experiments I and II.

The calculation of the mean percentage of non-hatched eggs among

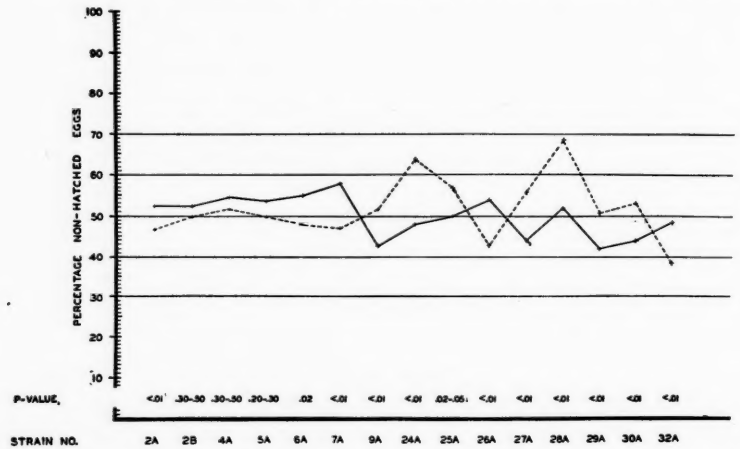

Fig. 5. Mean percentage of non-hatched eggs observed in strains 2A to 9A and 24A to 32A. ———connects mean values observed during the first half of the egg laying period. - - - - - - connects mean values observed during the second half of the egg laying period. The P-values (for the individual strains) entered on the figure were obtained by chi square analyses of the difference observed between the first and the second halves of the egg laying period.

those deposited during the first half and among those deposited during the second half of the egg laying period in experiment I are presented in Fig. 5. As can be seen from the figure, the variation between the strains is much higher for the last part of the egg laying period than for the first part. A similar analysis of the data observed for the strains investigated in experiment II is shown in Fig. 6. Here also it can be easily observed that the variation is much less between strains for the first three-day period than it is for the rest of the egg laying period.

Chi square analyses (carried out for each strain individually) of the difference observed between first and second half of the egg laying

period were performed. The P values obtained are listed on Figs. 5 and 6. They indicate how frequently such differences as observed would be expected due to chance alone. It can be seen that among the unrelated strains (Fig. 5), eight decrease while seven increase in the percentage of non-hatched eggs after the first three days. The differences observed between the first three days and the rest of the egg laying period are significant in ten strains, while three strains do not vary more than is expected due to chance and two of the strains fall in the doubtful range.

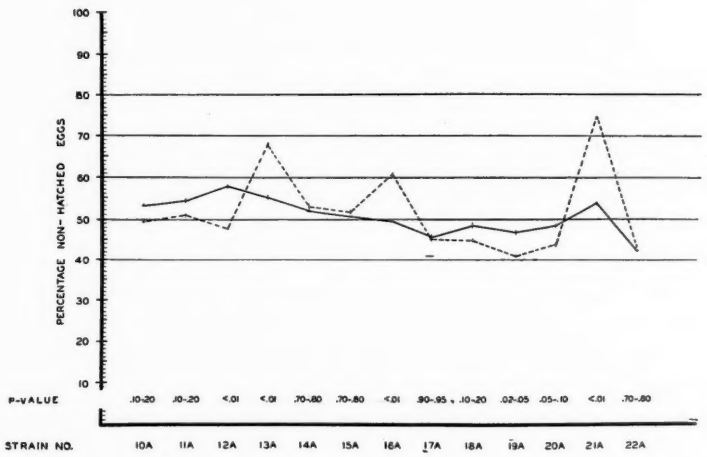

Fig. 6. Mean percentage of non-hatched eggs observed in strains 10A to 22A. ——— connects mean values observed during the first half of the egg laying period. - - - - - - connects mean values observed during the second half of the egg laying period. The P-values entered on the figure were obtained by chi square analyses of the differences observed (for each strain individually) between the first and second halves of the egg laying period.

Among the related strains (Fig. 6), it can be seen that six increase while seven decrease in their percentages of non-hatched eggs from the first part to the last part of the egg laying period. The variation between the first and the last part of the egg laying period is significant in four of the strains, no more than expected due to chance alone in eight strains, while one strain falls in the doubtful range. In the ten cases of the unrelated strains, where a significant variation is observed, six strains have increased and four strains decreased in the percentage of non-hatched eggs. Of the four related strains exhibiting significant variation, three

TABLE 2. *The percentages of non-hatched eggs observed in the first half and the second half of the egg laying period in the control series.*

Strain No.	13 A	17 A	19 A	20 A	21 A	22 A	24 A	28 A
First half of egg laying period	1,35	9,96	1,93	1,19	12,79	1,12	4,38	3,83
Second half of egg laying period	1,70	7,88	1,23	0,79	9,26	0,52	2,71	1,39
Level of significance of the difference	0,50 — 0,70	0,10 — 0,20	0,30 — 0,50	0,70 — 0,80	< 0,01	0,30 — 0,50	0,05 — 0,10	< 0,01

strains have increased and one decreased in the percentage of non-hatched eggs.

The hatchability observed in the control series was analysed with regard to the percentage of non-hatched eggs in the first half and the second half of the egg laying period. Table 2 shows that all strains except one gave fewer non-hatched eggs in the latter part than in the first part of the egg laying period. In only two strains are the differences significant and in both cases more eggs hatched in the second than in the first half of the period.

THE VARIATION WITHIN STRAINS.

A chi square analysis of the variance within the strains in repeat A, B, and C (experiment IV) was carried out and is presented in Table 3. It may be seen that while significant variation was observed only within one strain in repeat A and within two strains in repeat B, the variation was significant in five out of eight strains in repeat C. This indicates that a much higher homogeneity within strains is found when only eggs fertilized and deposited during the first three days of the egg laying period are counted.

Hence, the analyses of the within strain variation for the data observed in experiments I and II were carried out separately for the total, the first, and the second half of the egg laying period. The results obtained by chi square analyses of the data observed in experiment I are listed in Table 4, while Table 5 lists the results obtained by similar treatment of the data observed in experiment II. For the total egg laying period in experiment I it can be seen that four of the strains have a larger variation within strains than would be expected due to chance alone, ten strains have a variation no greater than expected, while three strains fall in the doubtful range. Another interesting thing to be noted

TABLE 3. *Homogeneity tests of variation within strains in experiment IV. The chi squares and the corresponding P-values are listed for the data obtained in repeat A, B and C. The chi squares obtained in C have the same degrees of freedom as those obtained in B. Below the table the strains are summarized according to their level of significance.*

	Repeat A			Repeat B			Repeat C	
Strain No.	Degrees of Freedom	χ^2	P	Degrees of Freedom	χ^2	P	χ^2	P
10A	4	11,72	0,01—0,02	4	2,44	0,50—0,70	8,60	0,05—0,10
11A	4	21,81	< 0,01	4	1,62	0,80—0,90	17,41	< 0,01
13A	3	2,72	0,30—0,50	4	8,25	0,05—0,10	24,91	< 0,01
16A	4	4,26	0,30—0,50	4	5,94	0,20—0,30	4,24	0,30—0,50
17A	4	3,71	0,30—0,50	4	18,36	< 0,01	36,12	< 0,01
19A	4	6,67	0,10—0,20	4	1,64	0,80—0,90	9,20	0,05—0,10
21A	4	13,13	0,01—0,02	4	20,81	< 0,01	110,04	< 0,01
22A	3	1,32	0,70—0,80	4	9,50	0,02—0,05	21,28	< 0,01
Significant variation			1			2		5
Doubtful variation			2			1		0
Insignificant variation			5			5		3

TABLE 4. *Homogeneity tests of variation within strains in experiment I. The chi squares and the corresponding P-values are listed for the eggs deposited during the whole period of egg laying, for eggs laid during the first three days, and for eggs deposited during the second part of the egg laying period. Below the table the strains are summarized according to their level of significance.*

Strain	Degrees of	Total		First Half		Second Half	
No.	Freedom	χ^2	P	χ^2	P	χ^2	P
2A	8	22,68	< 0,01	12,27	0,10—0,20	20,03	0,01—0,02
2B	9	16,17	0,05—0,10	7,00	0,50—0,70	13,62	0,10—0,20
4A	7	29,16	< 0,01	9,64	0,20—0,30	32,39	< 0,01
5A	9	16,25	0,05—0,10	5,58	0,70—0,80	26,44	< 0,01
6A	9	9,52	0,30—0,50	11,04	0,20—0,30	15,53	0,05—0,10
7A	9	8,35	0,50	18,33	0,02—0,05	14,45	0,10—0,20
9A	4	10,47	0,02—0,05	12,09	0,01—0,02	4,67	0,30—0,50
23A	9	13,25	0,10—0,20	13,25	0,10—0,20		
24A	8	16,34	0,02—0,05	13,00	0,10—0,20	43,25	< 0,01
25A	6	14,24	0,02—0,05	5,48	0,30—0,50	25,17	< 0,01
26A	7	7,63	0,30—0,50	7,58	0,30—0,50	6,78	0,30—0,50
27A	9	28,32	< 0,01	9,95	0,30—0,50	40,86	< 0,01
28A	9	66,84	< 0,01	31,38	< 0,01	60,80	< 0,01
29A	9	5,74	0,70—0,80	10,63	0,30	16,14	0,05—0,10
30A	9	14,52	0,10	6,56	0,50—0,70	30,87	< 0,01
31A	9	8,99	0,30—0,50	8,99	0,30—0,50		
32A	9	14,59	0,10	7,76	0,50—0,70	20,79	0,01—0,02
Significant variation			4		1		7
Doubtful variation			3		2		2
Insignificant variation			10		14		6

TABLE 5. *Homogeneity tests of variation within strains in experiment II. The chi squares and the corresponding P-values are listed for the eggs deposited during the whole period of egg laying, for eggs laid during the first three days, and for eggs deposited during the second part of the egg laying period. Below the table the strains are summarized according to their level of significance.*

Strain No.	Degrees of Freedom	Total χ^2	Total P	First Half χ^2	First Half P	Second Half χ^2	Second Half P
10A	9	7,21	0,50—0,70	9,01	0,30—0,50	8,32	0,50
11A	9	4,85	0,80—0,90	14,28	0,10—0,20	8,88	0,30—0,50
12A	9	14,04	0,10—0,20	8,71	0,30—0,50	15,27	0,05—0,10
13A	9	47,24	< 0,01	36,67	< 0,01	46,39	< 0,01
14A	7	100,61	< 0,01	74,79	< 0,01	40,61	< 0,01
15A	9	23,00	< 0,01	21,76	< 0,01	8,77	0,30—0,50
16A	9	33,59	< 0,01	12,20	0,20—0,30	40,38	< 0,01
17A	8	29,80	< 0,01	10,52	0,20—0,30	20,08	0,01
18A	9	14,54	0,10—0,20	8,20	0,50—0,70	21,77	< 0,01
19A	7	8,30	0,30—0,50	6,72	0,30—0,50	9,67	0,20—0,30
20A	9	14,98	0,05—0,10	18,50	0,02—0,05	23,21	< 0,01
21A	8	29,16	< 0,01	22,02	< 0,01	12,42	0,10—0,20
22A	5	8,88	0,10—0,20	2,16	0,80—0,90	7,98	0,10—0,20
Significant variation			6		4		6
Doubtful variation			0		1		0
Insignificant variation			7		8		7

is that, although 2A and 2B are of the same Oregon stock, the variation within the highly inbred strain No. 2B is no greater than that expected due to chance alone, while the variation within the other strain, 2A, which has been maintained by mass transfer, is significantly greater.

When the first part of the egg laying period in experiment I is considered, a significant variation is found within only one strain, while for the last part of the egg laying period seven strains are found to vary significantly.

When the total egg laying period for the strains in experiment II is considered, it may be noted (Table 5) that six strains have a greater variation than expected due to chance alone and seven strains do not vary more than expected. When the first half of the egg laying period is considered, four strains show a significant variation, while in the last part of the egg laying period six strains vary thus.

It seems from this that a large part of the uncontrolled variation within strains may be eliminated by observing the hatchability of the eggs laid during the first three days after the sperms have been

TABLE 6. *Homogeneity tests of variation within strains in the control.*

Strain No.	Degrees of Freedom	Total χ^2	P
13A	8	6,416	0,50—0,70
17A	9	1.380,150	< 0,01
19A	9	42,935	< 0,01
20A	9	7,667	0,50—0,70
21A	6	51,524	< 0,01
22A	9	11,930	0,20—0,30
24A	8	15,807	0,02—0,05
28A	9	108,160	< 0,01

irradiated, and that conclusions regarding the mean sensitivity of given strains should be drawn on the basis of these data.

The statistical analysis of the data for the untreated strains in experiment III is shown in Table 6. Four of the strains exhibit a highly significant variation, three do not vary more than would be expected due to chance, while one is in the doubtful class. The strains varying significantly were equally distributed between the strains giving high and those giving low percentage of non-hatched eggs after the sperms had been treated with X-rays. The strains giving a high percentage of non-hatched eggs were numbers 13A, 21A, 24A and 28A. Among these four, the grand mean of non-hatched eggs for the treated series was 61,12 per cent, while the grand mean for the control series was 4,45 per cent. In the group giving the lowest percentage of non-hatchability after X-ray treatment were strains number 17A, 19A, 20A and 22A. The grand mean of non-hatched eggs for this series was 44,05 per cent, while the grand mean in the corresponding control series was 3,20 per cent. A significance test of the difference between these two control series gave a chi square value of 0,102 which corresponds to a probability point between 0,70 and 0,80.

Although some of the within strain variations in the control series were greater than could be attributed to chance alone, these cases seemed to be distributed randomly between the groups (high and low when treated). The variance between them was insignificant and indicated, therefore, that the factors determining the level of non-hatchability of eggs without treatment have a very small effect on the differences in the frequencies of non-hatched eggs observed after sperm irradiation.

THE VARIATION BETWEEN STRAINS.

A chi square test of the data for the total and for the last part of the egg laying period indicated highly significant between strains variation, both for experiment I and experiment II. However, it was pointed out earlier that some of the uncontrolled within strain variation may be eliminated if only the eggs that are laid during the first three days are taken into account. It may also be seen from Figs. 4, 5 and 6 that the variation between strains is much less when the first part of the egg laying period is considered. A test of the variation for this period in experiment I gave:

Total chi square	282,25	with	155	degrees of	freedom
Between strains chi square	91,72	»	16	»	»
Within » » »	190,53	»	139	»	»

This very high chi square between strains strongly suggests a real difference in sensitivity of the sperms of different strains, even though the within chi square is somewhat higher than would be likely to occur due to chance alone.

A similar test of the variation for the strains in experiment II gave:

Total chi square	296,77	with	119	degrees of	freedom
Between strains chi square	51,23	»	12	»	»
Within » » »	245,54	»	107	»	»

The strains in experiment I, as well as those in experiment II, were not irradiated on the same day but divided into groups which were treated on different days. Thus, part of the between strains variation might be due to treatment differences. It is, therefore, necessary to determine what differences, if any, are attributable to treatment differences. If such differences are found to exist it would, of course, be desirable to determine their causes and to eliminate them if possible.

As previously noted, repeats A and B in experiment IV were carried out to test the difference between treatments. Fig. 4 shows that there is a fairly good agreement between the data observed in repeat A and repeat B. P-values, indicating how frequently a difference as great or greater than that observed between individual strains in repeat A and B might be expected by chance, were obtained by chi square analysis. These values are listed in Fig. 4. It can be seen that the differences between one X-ray treatment and another show a variation no more than would be expected due to chance, except for strain number 22A, which shows a significant variation between treatments.

The chi square between treatments within strains is 17,62 with 8 degrees of freedom, which corresponds to a P value between 0,02 and 0,05. This suggests some difference between treatments, although it may be noted that the major contribution to treatment effects is by strain 22A. If this strain is not included in the calculations, the chi square is 6,70 which is less than the degrees of freedom (7), corresponding to a P value between 0,30 and 0,50, and not indicating any significant effect of treatments. It may well be that the significant effect in the case of strain 22A is due to segregation within the strain of genes affecting sensitivity to radiation. It is, of course, possible that the significant difference observed in this strain between treatments may be due to a rare chance occurrence. Despite the absence of significant overall variation between males of this strain, it is very likely that some variation between individual males remains, and one may, therefore, when a small number such as five males is collected, happen occasionally to select one giving a high percentage of dominant lethals.

When the chi square between strains within treatments is considered, an entirely different situation is revealed. The chi square, due to differences between strains within treatments, is 32,144 with 7 degrees of freedom for the first treatment and 48,165 with 7 degrees of freedom for the second. Both these chi squares, as well as their sum, are of course very highly significant with $P < 0,01$. If strain 22A is eliminated, the between strain within treatments chi square is 36,196 with 6 degrees of freedom for the first and 19,455 with 6 degrees of freedom for the second treatment. Both these chi squares are also highly significant.

Although the between strains chi square cannot be taken at face value as long as there is greater than random variation between treatments, which suggests some uncontrolled variation, the relatively slight evidence for treatment effect in connection with the exceedingly low P value relating to strain differences indicates very strongly that the strain difference is a real one.

If it can be shown that significant correlation exists between the same strains tested at different times, little doubt would remain that the observed variation between strains cannot be explained on the basis of uncontrolled variation. When all strains are included in the calculations, a correlation coefficient of 0,7066 with 6 degrees of freedom is obtained, which is identical with the tabular value for the five per cent level of significance. A higher significance is obtained when strain number 22A is excluded from the calculations. In that case a correlation coefficient

of 0,8079 with 5 degrees of freedom is obtained, the tabular value for the five per cent level of significance being 0,7545.

It appeared worth while to make a simple analysis of variance of the data collected for the first half of the egg laying period in experiment I and in experiment II. It is not necessary to transform the data since the hatchability for all strains does not vary much from 0,5. We may first see if there is a significant mean square between strains in terms of that between males within strains. In experiment I this ratio is 3,919 with 16/139 degrees of freedom, which is highly significant. The question presents itself, however, as to whether the between strains difference may be merely a reflection of uncontrolled environmental differences between treatments carried out on different dates. To test this, we may compare the between treatments mean square with the mean square between strains within treatments. The ratio is 1,949 with 7/9 degrees of freedom and is not significant. In analysing experiment II in the same manner, a ratio of between strains to between males within strains of 2,596 with 12/107 degrees of freedom is obtained, which is significant. In this case, the ratio of between treatments to between strains mean squares is 5,746 with 4/8 degrees of freedom, which is in the doubtful range between $P = 0{,}05$ and $P = 0{,}01$.

The higher significance of the strain difference in experiment I, as compared to that of experiment II, is consistent with the absence of relationship between strains in experiment I and the close relationship in experiment II.

It was mentioned previously that some strains were maintained by regular mass transfer, while others were maintained by brother-sister matings. When the variance ratio is computed for the homogeneity within strains, maintained by these two methods of breeding, an F value of 1,664 with 181/65 degrees of freedom is obtained. The tabulated value for one per cent of significance with 200/65 degrees of freedom is 1,64.

The hypothesis then may be proposed that there is some kind of segregation affecting the percentage of hatched eggs so that individual flies from a given strain or flies from different strains will produce sperms with a different percentage of dominant lethals. Thus, one may suppose that some strains have genotypes extra-sensitive to irradiation, and that there are both fairly homogeneous and heterogeneous strains in this respect. This hypothesis will explain the high variation observed between strains as well as the within strain variation. The incidence and variability of each strain under treatment and control conditions are listed in Table 7. Independence of spontaneous and induced lethality of

TABLE 7. *Incidence and variability of each strain under treatment and control conditions.*

Strain No.	Incidence		Variability	
	Treatment	Control	Treatment	Control
13A	54,96	1,35	39,47	2,33
17A	45,29	9,96	23,15	84,91
19A	46,35	1,93	20,00	7,83
20A	48,10	1,19	19,24	2,78
21A	53,65	12,79	42,10	21,43
22A	42,12	1,12	12,46	3,42
24A	47,79	4,38	26,97	7,45
28A	51,25	3,83	30,66	19,17

the eggs will explain also why some strains with significant variation under treatment do not always show significant variation in the control series.

DISCUSSION.

The different experimental methods used by different investigators who have studied the hatchability of eggs fertilized by irradiated *Drosophila* males may well account for some of the conflicts among the published data. Thus, the method of egg counting employed by the author was different from that described for similar experiments reported in the literature. Some investigators have reported that they count the eggs only after hatching when the number of eggs hatched and not hatched are recorded. Other authors report that they count all the eggs laid and after hatching they count eggs which failed to hatch and obtain the number of hatched eggs by subtraction. Both these methods are of course subject to errors leading to biased results. The present author has noticed that when eggs are deposited in clusters very often some of the empty egg shells are hidden underneath or in between the non-hatching eggs. The larvae carry a few empty egg shells with them and bury them in the food medium, and other times the yeast growth may be so heavy that the eggs are impossible to find. To avoid errors of this kind, throughout this investigation the eggs were always counted when first collected and both non-hatched and hatched eggs were counted after incubation.

In a few cases abnormal eggs were obtained. They had a transparent pinkish colour with a clear centre. All eggs of this type could be readily counted when the eggs were collected, but after incubation the recount of the eggs was equivocal due to the transparent look of the chorion which sometimes made it difficult to distinguish it from the

medium or from the first instar exuviae. The unhatched eggs of this abnormal type often sank into the medium as though their specific gravity was much higher than that of normal eggs, and it is likely that some sank so far that they could no longer be seen. The hatchability for this type of eggs, however, seemed to be the same as for normal eggs. Due to the uncertainty of the recount of these eggs, batches with abnormal eggs were excluded from the data. Otherwise, no selection of data was practiced except the elimination of completely sterile cultures, as well as of the data from strain 8A as previously mentioned.

The author (1949) discussed at some length all the precautions taken in order to have as few variables as possible. He also pointed out that the sensitivity to X-rays increased with the age of the sperm and also with the length of time taken in developing from egg to imago by the irradiated individuals. It was thought possible that increased time for development might denote increased age of the sperms, since the formation of mature spermatozoa is initiated in the pupal stage around three days before emergence of the pupae (BODENSTEIN, 1950). Therefore, in experiments described in this paper, additional precautions were taken to utilize for irradiation only sperms of approximately the same age. This included the selection of flies that took the same time in developing from egg to imago, and the storage of these selected individuals for exactly the same number of days from emergence to the time of irradiation. Thus, in the experiments here reported, sperms were irradiated from males having developed in approximately ten days and stored for approximately four and one-half days before irradiation. It would be preferable if these two variables affecting age were stated in tests of this sort rather than merely the time between emergence and irradiation as is usual.

BONNIER and LÜNING (1950) found that there was a decline in hatchability of eggs with increase in the time that the sperms were stored from irradiation to fertilization. Especially pronounced was the drop in hatchability after the third day. The data published in the present paper showed that when the hatchability of eggs fertilized on the three first days after irradiation was compared with the hatchability of eggs fertilized on the fourth to the sixth day after irradiation, in fourteen strains the variation in hatchability was insignificant, while in another fourteen strains the variation was significant. Of the last fourteen strains, nine exhibited a significant decrease in hatchability while five strains had an increase in hatchability between the two periods.

IVES (1950) studied the mutation rate of sex linked lethals in sperms

from males homozygous for *hi*, which is a gene for high mutation rate located in the second chromosome. The males were transferred to new sets of females twice weekly for a period of four weeks. He found that three sets of males consistently gave a decrease in percentage lethals from the first to the fourth week. In a fourth set of males, the frequency in the last three weeks was higher than in the first week. IVES explained the decrease in lethals observed in the former case as partly due to germinal selection and partly due to the action of *hi*. It was believed that *hi* produced most of its effect while the males were young and that it ceased to produce this particular effect after the males had emerged as adults. In the latter case it was believed that a modifying gene prolonged the action of *hi* so that the lethals produced were at least of the frequency of those lost by germinal selection. It was also shown by IVES that *hi* produced inversions at a minimum rate of approximately one in 400 germ cells.

DUBOVSKY (1935) investigated the number of recessive lethal mutations induced in the X-chromosome of different strains of *Drosophila melanogaster* when the males had been treated with 4500 r-units of X-rays. He selected for analysis the strains Florida and Crimean, giving the lowest, and Pyatigorsk, giving the highest, percentage lethals, and stated that the difference between them was statistically significant. From the data published for all the strains it was possible to make a chi square test which gave a between strains chi square of 14,668 with 5 degrees of freedom. The probability level is between 0,01 and 0,02. However, no information was given to allow the computation of the within strain chi square so that the conclusion mentioned is somewhat doubtful.

DEMEREC and FANO (1944) came to the conclusion that different stocks of flies produce approximately the same frequency of dominant lethals when they are given equal X-ray treatments.

What seem to be discrepancies in the findings by different authors have also occurred in the experiments described here. Thus, the ageing of the sperm between the time of irradiation and the time of fertilization has been reported to have no effect in some strains, while in other strains a decrease in the frequency of induced dominant lethals was observed, and in still other strains the frequency was increased. Many of the strains investigated gave approximately the same mean percentage of dominant lethals. However, both a chi square analysis and the analysis of variance of the strains in experiments I and II indicated significant differences between them. The chi square analyses of the data from the

two repeats A and B indicated also a significant difference between strains, and the correlation between the same strains irradiated at different times is a further indication that these strain differences cannot be attributed to treatment differences or other uncontrolled variation.

The question remains as to how genetic differences between strains result in differences in the proportion of dominant lethals induced by a given dosage of X-rays. Dominant lethals probably consist chiefly of chromosomal breakages that have led either to dicentric and acentric chromosomes, sister strand union, deletions, asymmetrical interchanges, or simply broken ends that remain open (CATCHESIDE, 1948; MULLER, 1950). MOHR (1925) points out that no mutations have ever been found that are gametic lethals in animals. This was confirmed by experiments performed by MULLER and SETTLES (1927).

The difference in sensitivity of different strains may then result from one of the following possibilities:

(1) The frequencies of actual or potential breaks induced by X-ray irradiation may differ in different strains.

(2) The probability that a potential break will become an actual break at fertilization may differ in different strains.

(3) The probability that a break will reconstitute the original combination of chromosome parts may differ in different strains.

Probably no data are available that would permit one to discriminate between these possibilities except in a highly tentative manner.

Regarding the first possibility, it is known that environmental conditions, notably that of the oxygen tension, affect the frequency of recessive and dominant lethals in *Drosophila* (BAKER and SGOURAKIS, 1950). Therefore, it would seem probable that genetic differences could affect the mutation rate indirectly by altering the chromosomal environment. SWANSON (1947) showed that pre- and post-treatment with infrared light increases the frequency of chromosomal re-arrangements in *Tradescantia*, whereas KAUFMANN, HOLLAENDER and GAY (1946) find that only pre-treatment exerts an analogous effect in *Drosophila* sperms.

MULLER (1940) shows that in *Drosophila* sperms the joining of broken ends does not start until fertilization takes place. This is supported by the fact that the proportion of chromosome alterations (No. of II—III translocations) due to irradiation are not markedly different whether the flies are mated immediately or 30 days after irradiation. CATCHESIDE (1948) in his review shows that in plant materials some joining may take place already during irradiation. Thus, an erroneous opinion may be formed if observations made on one organism are un-

critically transferred to another. However, there is no doubt that many environmental changes (such as oxygen tension) may influence the pollen grains and the sperm cells (although these are in stages of different metabolic activity) in the same direction.

The second possibility mentioned is difficult to discuss, inasmuch as there is little or no evidence bearing either on the question as to whether potential or actual breaks are produced by X-rays in *Drosophila* sperm or on the nature of potential breaks, if they are in fact produced.

With regard to possibility three, a number of points may be discussed. In view of MULLER's conclusion mentioned above, that chromosomes restitute or engage in new combination only at or after the time of fertilization, the broken ends must be thought of as having mutually adhesive and perhaps also mutually attractive tendencies at this time. If the two ends of a given break are within a certain distance of each other, the original chromosome may be restituted. The distance the broken ends move apart should depend, in part at least, on the tension in the chromosome and the space available for movement.

There is also the possibility that the energy reserves of the sperm may decrease with time, and that the tendency of broken ends to attract each other so as to come together in the newly fertilized egg may depend on energy reserves. If the reserves in the sperm have been depleted by time, restitution may be delayed until the egg cytoplasm replaces the lost reserves, at which time the chromosomes would have had time to move farther from their original positions. At any rate, whatever effect is brought about by age might also be brought about, to some degree, by genotype; the latter might reasonably be expected to affect the speed at which changes occur with time.

It is now necessary to consider the various types of irregularities observed, both with respect to their explanation and their possible bearing on the hypotheses considered above.

Assuming that equal doses produce the same number of breaks and that the joining of broken ends is completely at random, one would expect to find equal frequencies of dominant lethals induced in different wild type stocks. If, therefore, significant differences are observed, this may be due to genetic differences governing one or more of the processes described above. It may be that some stocks contain mutator genes which increase the sensitivity to X-ray produced breakages. This may increase the frequency of gametes leading to inviable zygotes. In both cases, qualitative differences between strains would be reproduc-

ible. The observed within strain variation may thus be due in part to genetic segregation in these strains. As mentioned earlier, some of the strains used have been maintained by regular mass transfer, while other strains have been inbred by brother-sister matings to varying degrees. The variance ratio of the within strain variation of the strains inbred by mass transfer to those inbred by brother-sister matings shows that the difference in homogeneity of the strains, maintained by these two breeding methods, was significant.

The ageing of the broken ends is more difficult to explain. The suggestion made by BONNIER and LÜNING that the broken ends move apart could explain the decrease in hatchability. However, the simultaneous observed increase in hatchability in other strains would then have to be explained on the basis that the broken ends move closer together. Another explanation may be that the affinity of broken ends from the same chromosome increases with the lapse of time in some strains, while the reverse is true in other strains. To explain this on a genetical basis, one would have to assume modifying genes of the type suggested by IVES, so that the genes controlling the frequency of dominant lethals have to start acting or stop acting at certain times. This seems so unlikely that a simpler hypothesis would be welcome. If it is assumed that the metabolic processes going on in the sperm and other environmental factors are responsible for the ageing effect observed, one would not expect to be able to reproduce it always. This explanation finds some support in the previously described experiments.

It is realized that some variation is expected due to the mating frequency. In the first mating, the female will receive sperm developed already in the pupal stage and in later matings she will receive sperm matured at a time closer to the time of irradiation. Eggs deposited on the first day will then be fertilized by the oldest sperm and on succeeding days the eggs will be fertilized by sperms of mixed age. KAUFMANN and DEMEREC (1942) showed by using adequate marker genes that sperms from different matings mix in the female receptacles and that chance and the relative frequency of the different types of sperms determine what kind of sperm will fertilize the egg.

SUMMARY.

Males from 31 strains of *Drosophila melanogaster* were tested in regard to their sensitivity to the induction of dominant lethal mutations by 2300 r-units of X-ray. In one experiment, 17 unrelated strains, and,

in another experiment, 13 related strains were tested. Nine strains were tested in a third experiment for the frequency of spontaneous dominant lethals present. A fourth experiment was carried out in order to test the variation from one day's X-ray treatment to another, and also in order to test the variation in hatchability between eggs laid on the first to the third days and on the fourth to the sixth days after irradiation.

It was found that 14 strains varied insignificantly and another 14 strains varied significantly in their hatchability between the two three-day egg laying periods. Of the 14 strains varying significantly, nine showed a decrease and five an increase in hatchability in the second period.

The within strain variation was higher in the second egg laying period. Thus, five strains exhibited a significant within strain variation in the first three days of egg laying, while 13 strains varied thus in the second three-day period.

The conclusions regarding the sensitivity of the different strains to the induction of X-ray induced dominant lethal mutations were based on the data observed during the first three days after irradiation. It was found that the 13 related strains (differing only in their fourth chromosome) in experiment II varied significantly, as did also, and to a greater degree, the 17 unrelated strains in experiment I.

CATCHESIDE and LEA (1945) pointed out that reports from different authors appeared to be in disagreement with each other when a comparison was made of the rates of dominant lethals induced by equal doses of X-rays. The results of the present investigation suggest that part of these disagreements may be due to differences in period of egg laying and differences in genetic constitution of the stocks used. Other probable reasons are: differences in method of egg counting, differences in time of development of the flies irradiated and differences in age of flies when irradiated.

The experiments reported here are based on procedures minimizing such sources of variation and demonstrate that genetic differences in the sensitivity to induction of dominant lethal mutations by X-rays exist between unrelated strains of *Drosophila melanogaster*.

Acknowledgements. — I am particularly indebted to Dr. EVERETT R. DEMPSTER, Division of Genetics, University of California, Berkeley, for his constant interest and advice throughout the course of this investigation. I am also much indebted to Dr. MICHAEL LERNER, Division of

Poultry Husbandry, and Dr. CURT STERN, Department of Zoology, University of California, Berkeley, for many suggestions in the preparation of this manuscript.

Thanks are also expressed to all those listed in Table 1, who have contributed flies.

I wish to express my gratitude to Dr. LAURENCE SEARS, Berkeley, California, for financial support received through him, to Oslo University, Norway, for fellowships received 1948—1951, and to the United States Educational Foundation in Norway for a U. S. Government Travel Grant under the Fulbright programme.

This work was done in the Division of Genetics, University of California, Berkeley, California.

LITERATURE CITED.

1. BAKER, W. K. and SGOURAKIS, ELIZABETH. 1950. The effect of oxygen concentration on the rate of X-ray induced mutations in *Drosophila*. — Proc. Nat. Acad. Sci. 36: 176—184.
2. BODENSTEIN, D. 1950. The postembryonic development of *Drosophila*. — Biology of *Drosophila*, ch. 4: 275—367. New York; John Wiley & Sons, Inc.
3. BONNIER, G. and LÜNING, K. G. 1950. X-ray induced dominant lethals in *Drosophila melanogaster*. — Hereditas XXXVI: 445—456.
4. CATCHESIDE, D. G. 1948. Genetic effects of radiations. — Advances in genetics, vol. II: 271—358. New York; Academic Press, Inc.
5. CATCHESIDE, D. G. and LEA, D. E. 1945. The rate of induction of dominant lethals in *Drosophila melanogaster* sperm by X-rays. — Journ. Genet. 47: 1—9.
6. DEMEREC, M. and FANO, U. 1944. Frequency of dominant lethals induced by radiation in sperms of *Drosophila melanogaster*. — Genetics 29: 348—360.
7. DUBOVSKIJ, N. V. 1935. On the question of the comparative mutability of stocks of *Drosophila melanogaster* of different origins. — C. R. (Dokl.) Acad. Sci. U. R. S. S. 4 (9): 95—97. Acad. Nauk. Dok.
8. IVES, P. T. 1950. The importance of mutation rate genes in evolution. — Evolution 4: 236—252.
9. KAUFMANN, B. P. and DEMEREC, M. 1942. Utilization of sperm by the female *Drosophila melanogaster*. — Amer. Nat. 76: 445—469.
10. KAUFMANN, B. P., HOLLAENDER, A. and GAY, H. 1946. Modification of the frequency of chromosomal rearrangements induced by X-rays in *Drosophila*. I. Use of near infrared radiation. — Genetics 31: 349—367.
11. MOHR, O. L. 1925. Bemerkungen über das Verhalten gametischer Letalfaktoren bei Tieren und bei Pflanzen. — Avhandl. utg. av d. Norsk. Vid. Ak. i Oslo. I. M.-N. kl. 1925, 5: 8 pp.

12. MULLER, H. J. 1927. Artificial transmutation of the gene. — Science 66: 84—87.
13. — 1940. An analysis of the process of structural change in chromosomes of *Drosophila*. — Journ. Genet. 40: 1—66.
14. — 1950. Some present problems in the genetic effects of radiation. — Journ. Cell. and Compar. Physiol. 35, supplement 1: 9—70.
15. MULLER, H. J. and SETTLES, F. 1927. The non-functioning of the genes in spermatozoa. — Zeitschr. f. ind. Abst.- u. Ver. 43: 285—312.
16. STRÖMNÆS, Ö. 1949. The production of dominant lethals with X-rays in aged *Drosophila melanogaster* sperm. — Genetics 34: 462—474.
17. SWANSON, C. P. 1947. The effect of infrared treatment on the production of X-ray induced changes in the chromosomes of *Tradescantia*. (Abstr.) — Amer. Journ. Bot. 34: 590.

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ABSTRACTS

BERTIL RASMUSON: Ether susceptibility of two stocks of *Drosophila melanogaster*.

In an investigation concerning selection of certain bristle characters of *Drosophila melanogaster*, the animals in one selection line proved to wake up from ether narcosis much quicker than those in the other lines. This selection line had been cultured with two females and two males for about 50 generations when this behaviour was observed. The line was characterized after three observed generations by the same fast recovery time from the narcosis applied to both females and males. The cause of this characteristic must therefore be inherited. A Florida wild-type strain, which had been in culture for about 30 years and in selection experiments proved to be almost isogeneous, was used as a control.

It proved to be more suitable in the experiments to study either the time to anaesthetize the animals or the time to kill them by anaesthetization, instead of the recovery time. This permits more accurate measurements of the time factor and of the ether dose. Several different methods have been tried. Up till now the best method has been to use a glass tube (5 cm. in diameter and 25 cm. long), closed at both ends with rubber bungs. In the middle of the tube is fixed a rust-free steel circular membrane of fine mesh to divide the tube into two equal parts. On each side of the membrane is a hole in the tube through which the flies can be admitted after a certain amount of ether has been allowed to evaporate. With this method there is naturally a rather great variation of the dose, but on the other hand it is known that the dose is exactly the same on each side of the membrane. In each experiment comparisons can be made between two groups of animals, for example females from a normal strain with females from a sensitive strain, animals from the same strain but of different ages, progeny of reciprocal crossings, and so on. Every group studied consisted of 20 animals, and measurements were made every 15 seconds. Every experiment was repeated several times. The temperature was + 20° C. (Fig. 1).

The curves show that about three hours after hatching the difference between animals from the normal and from the sensitive strains is rather small, and that this difference increases to a maximum value between 12 and 24 hours after hatching. This value is more or less constant up to at least 144 hours. In some cases the curves show a peak at an age of 12 hours, a behaviour which will be studied in greater detail. In almost every case the males are more sensitive than the females.

Differences of about the same order are found in the progeny after reciprocal crossings between normal and sensitive animals: F_1 daughters of a sensitive mother and a normal father are anaesthetized faster than daughters of the reciprocal crossing for the same dose. The daughters of these reciprocal crossings have identical genotypes but cytoplasm of different origin, so the differences must depend on a maternal effect.

Another experiment was started to find out if those animals which were anaesthetized quickest in one group, also showed the same effect in a second

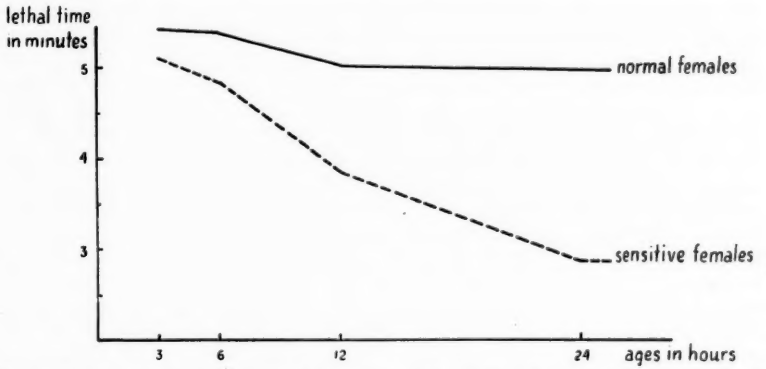

Fig. 1. Curves showing averages for normal and sensitive females for one series of experiments. Dose: about 30 volume per cent.

anaesthetization. To test this, a separating-funnel was used through which the animals could be passed out at certain intervals. The results showed a very weak correlation between the first and second anaesthetization, but it was unexpected that in every case the averages of the second anaesthetization were higher than those of the first. Thus the first anaesthetization has induced a certain transitory resistance: for a normal female the resistance has come to a maximum about 8 hours after the first anaesthetization, for sensitive females after about 2 hours. After about 32 hours they have returned to their original state again.

After treatment with different nerve gases the normals and the sensitives show differences of the same order as with ether. Since these substances have been proved to block the choline esterase, a determination of the choline esterase content will be made. In addition, some other physiological investigations have been started.

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MARIANNE RASMUSON: Variation in inbred lines of *Drosophila melanogaster*.

Using the inbreeding lines described by RASMUSON (1949), a study was made on the effect of inbreeding upon variation of quantitative characters. The brother-sister-mating of these lines was continued, and after about the 60th inbreeding generation the counts of sternopleurals and abdominal bristles were taken up again and carried on for 10 generations. At this time only 5 of the 10 original lines were left. Thereafter the lines were crossed reciprocally with each other and the bristles were counted in F_1 and F_2. The intention is to make 5 repetitions of each cross, but the experiment is not yet ended, and thus the values given below are only preliminary. Bristle-counts have also been made on pair-cultures from 4 wild populations of different origin.

In order to be able to compare the variation of the different populations

and inbred lines the mean squares have in all cases been calculated after the original values of the counts have been transformed to the logarithmic scale, which procedure makes the variance independent of the mean. It is possible then also to compare the relative variation of the two sexes and the different types of bristles though their means are considerably different (sternopleurals 15—20; abdominal bristles, males 30—40, females 40—50). The following table gives the pooled mean squares within cultures and the degrees of freedom on which they are calculated. Usually the counts included 20 animals of each sex from every culture, and all cultures with less than 10 males or females have been discarded. In the latest inbreeding generations sometimes more than 20 animals were included in the counts, though never more than 40.

	Abd. b. ♀♀		Abd. b. ♂♂		Sternopl. ♀♀		Sternopl. ♂♂	
	M. s.	D. f.	M. s.	D. f.	M. s.	D. f.	M. s.	D. f.
Wild popul.	0,1102	855	0,1203	855	0,1559	855	0,1913	855
Inbreeding generations								
2— 5	0,1416	748	0,1563	737	0,1387	748	0,1947	737
6—10	0,1213	927	0,1317	932	0,1080	927	0,1325	932
11—15	0,1281	832	0,1136	843	0,1087	832	0,1323	843
16—20	0,1077	753	0,1173	733	0,0951	753	0,1356	733
60—70	0,1576	1327	0,1357	1348	0,1005	1327	0,1211	1348
60—70 without *b*	0,0897	986	0,0871	1006				
F_1	0,0818	945	0,0897	943	0,0904	945	0,1179	943
F_2	0,1641	1134	0,1795	1140	0,1567	1134	0,1876	1140

Between the wild populations there were only small differences in variation. During the inbreeding the variation of the sternopleurals decreased, but at least the males showed an increase during the first few generations. There was no great difference of variation between the lines. The variation of the abdominal bristles, however, showed very great dissimilarities. Some lines had their variability reduced, but some achieved during the inbreeding a variance which by far exceeded that of the wild populations. Only one of these lines was left after 60 generations, line *b*. The table shows the difference in mean variance with and without this line.

The variation in F_1 is very uniform and is the smallest found in the whole experiment, smaller than in the inbred lines themselves. The variation of F_2 is about equal to that of the wild populations for the sternopleurals, but seems to exceed these in what concerns the abdominal bristles.

Comparisons between the variances of the two sexes show that the males are usually more variable than the females. This applies both to the wild populations and F_2 where genetical differences contribute to the variation, and to the inbred lines and F_1 where the genetical differences are reduced to a minimum. The only exception is the female abdominal bristle-number, which in the late inbreeding generations is more variable than the male.

The variances of the two bristle-types suggest that the variation of sternopleurals, as a rule, is relatively greater than that of the abdominal bristles.

In the wild populations the differences are highly significant. During inbreeding the extremely high variances of the abdominal bristle-numbers arise in some lines and increase the mean variance of this type of bristles, but in F_1 the variation of sternopleurals is again at little higher. In F_2 there are no definite differences.

Selection for high and low numbers of sternopleurals has been undertaken from the 61st generation of line *b*, and for high and low numbers of abdominal bristles from the 64th generation of the same line. The selection for sternopleurals had no effect. In the abdominal bristle-number, however, selection caused a difference of 4 bristles between the means of the high and the low line. This difference is significant at the 0,01 level. The selection was continued for 13 and 9 generations, respectively, but the difference was obtained already in the first generation of selection. When selection ceased, or its direction was reversed, the means immediately changed again.

Mean (males + females) of line *b*	42,61
» » » » high selection line (13 gen.)	45,89
» » » » low » » (9 »)	41,82
» » » » back » from high line (1 gen.)	41,98
» » » » » » » low » (1 »)	45,42

The animals with the lowest number of abdominal bristles had a very poor fertility, which retarded the low selection. The most extreme animals of this line seemed to be quite sterile.

The behaviour of this line *b* suggests that some kind of delayed environmental effect rather than real genetic differences cause its great variability, although more detailed investigations are demanded on this point.

Institute of Genetics, University of Stockholm.

Literature cited.

1. RASMUSON, M. 1949. Inbreeding and selection on quantitative characters in *Drosophila*. — Proc. Eighth Int. Congr. Genet. (Hereditas, Suppl. Vol.), pp. 646—47.

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Utgivet den 18 augusti 1951.